RESPIRATORY FUNCTION STUDIES

IN

NORMAL AND BRONCHITIC RATS

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C O N T E N T S

INTRODUCTION

AND

AIM OF PRESENT STUDY

INTRODUCTION

The symptoms of chronic bronchitis and emphysema do not correlate well with histopathological changes in the lungs. Cabot (1927) found that in the lungs of twelve patients diagnosed clinically as suffering from pulmonary emphysema, there was no pathological evidence of emphysema in nine. Out of one hundred and fifty three pathological diagnoses, only seven was recognized during life. Christie (1952) recorded that of seventy two cases diagnosed at necropsy, a history of dyspnoea was mentioned only in thirty five. Monroe (1951) analysed one hundred and twenty nine autopsies in which emphysema was diagnosed either clinically or pathologically. He found that emphysema was omitted from the pathological report in one hundred and ten cases (85%). In fourteen cases (11%), advanced emphysema was found at necropsy, but no clinical diagnosis had been made and there was no record of dypsnoea in the case histories. In only five cases (4%) did antemortem and post-mortem diagnosis agree. Baldwin, Cournand and Richards (1949) studied in great detail a patient with severe respiratory functional disturbances over a period of many years, but could find little pathological changes, apart from fibrosis of the bronchiolar walls, at autopsy.

Two questions arise from the above observations. Is emphysema a clinical entity more allied to disturbances in function than alteration in structure

(Ogilvie,1959)? Do tissue changes have much to do with symptoms (Monroe,1951)?

There is no doubt that some of the discrepancies can be accounted for on the one hand by the loose clinical usage of the terms "bronchitis" and "emphysema" and, on the other, by the difficulty in demonstrating emphysema at post-mortem and the rather unreliable signs of emphysema pathologists have used. The difficulty in the clinical diagnosis of emphysema has been emphasized by Fletcher(1952). He demonstrated that there was considerable observer error in the assessment of physical signs of emphysema and suggested that objective tests of pulmonary function would be helpful in early diagnosis. Increase precision in pathological diagnosis of emphysema followed the introduction of improved techniques of post-mortem study of lungs first by Gough and Wentworth (1949) using paper-mounted whole lung sections, and more recently by Heard (1958) employing barium sulphate impregnation techniques. Thus, centrilobular emphysema was first recognized and its aetiological relationship to bronchial infection postulated by Gough (1952). The pathological definition and classification of emphysema in more accurate terms also become possible and has been the subjects of two recent symposia (Ciba Guest Symposium,1959. World Health Organization Committee on Chronic Cor Pulmonale, 1961). A World Health Organization Committee (1961) defined emphysema as a condition of the lung characterised by increase beyond the normal in the size of air spaces distal to the

terminal bronchiole with destruction of their walls.

Although the definition of emphysema on a pathological basis seems clear, the functional disturbances associated with the pathological changes still remain a subject of much debate. Fletcher, Hugh-Jones, McNicol and Pride (1963) in a recent study classified their patients into three categories - Primary Emphysema, Bronchitis with emphysema, and, Bronchitis without emphysema - on clinical and radiological grounds. They showed that there were distinct differences in the disturbances in function between the 'Emphysema' and 'Bronchitis' groups. Whereas right heart failure and alveolar hypoventilation were prominent features in the 'Bronchitis' group, the 'Emphysema' group had relatively normal blood gas tensions and an impaired carbon monoxide uptake. It would appear that functional changes which had hitherto been thought to indicate emphysema could be due to 'Bronchitis', and 'Emphysema' alone would be compatible with comparatively little disturbance in function.

One of the difficulties in correlating structural and functional changes in human bronchitis and emphysema lies in the uncertainty in the diagnosis of emphysema during life. Both clinical and radiological signs are unreliable unless the emphysema is advanced (Fletcher, 1952. Knott and Christie, 1951). It is therefore not easy to ascribe a particular functional disturbance to emphysema. The difficulty in correlation is

added to by the chronicity of the disease so that functional studies in a patient may have been done years before his death when histopathological details of the lungs become available. The characteristic patchy involvement of the lung parenchyma in emphysema makes lung biopsy a rather unsatisfactory procedure because a representative sample may not be obtained and, in any case, it gives no indication as to the extent of the disease. Besides, histological interpretation of biopsy material in which the size of the air spaces alone is probably the prime factor, is apt to be inaccurate. As there is also evidence that bronchitis and emphysema may produce different patterns of functional changes (Fletcher et. al.,1963), the frequent co-existence of the two conditions, although possibly implying aetiological inter-relationship, tends rather to obscure the functional disturbances related to each disease process.

It is probable that some of these difficulties may be overcome by experimental work on animals. The advantage here is that if functional studies can be carried out on animals, they may then be sacrificed and the functional data can be linked directly with histopathological findings. To do this, respiratory function in the normal animal should first be studied and its limits defined; then the pathological changes of bronchitis or emphysema, in increasing degrees of severity, induced, and the associated functional changes

determined. Such a study has hitherto been hampered by two major obstacles. The first has been the discovery that most laboratory animals suffered from some form of endemic bronchial disease so that suitable control animals are not available. The second has been the almost uniform failure to produce emphysema experimentally, although there is some indication that oxides of nitrogen may induce emphysema in the mice (Bates,1962).

It has been known that laboratory rats suffered from an endemic respiratory disease which is world-wide in distribution. This so called 'endemic pneumonia' manifests histologically as a peribronchiolitis with proliferation of goblet cells, increase bronchial secretions, lymphoid hyperplasia, aspiration of secretions with peribronchiolar lung damage and emphysema. The disease differed from human bronchitis only in the prominence of the lymphoid hyperplasia (Cruickshank,1948). Histological preparations of human and rat lungs showing the similarity in the pathological changes of bronchitis in the two species are shown in Figure 1.

Nelson (1946,1948), in a series of carefully conducted experiments, demonstrated that the endemic disease in rats could readily be transmitted to mice by nasal instillation of lung suspensions from diseased rats. The responsible agent was filterable and belonged to the pleuropneumonia-like group of organisms (PPLO) (Nelson,1948. Kleineberger-Nobel,1962). He showed that young rats became infected at a very early stage of life,

but possibly because of the transference of maternal antibodies, these young animals exhibited marked tolerance and were able to confine the infective agent to the upper respiratory passages. In time, however, the infection spread downwards to involve the bronchi and lungs. The severity of the disease seemed to increase with age; the disease had a low mortality but a high morbidity. Nelson (1951) subsequently showed that if pregnant rats were delivered by Caesarian section and the offspring weaned by hand, a colony of rats free from respiratory disease could be reared and maintained. Such rats are not free from all bacteria, but do not suffer from the endemic respiratory disease. They have therefore been designated 'Specific Pathogen Free' (S.P.F.). Their bronchi are healthy and this supports the view that the lymphoid hyperplasia in rats is not a species variant but directly related to the endemic disease.

The Department of Therapeutics, Queen's University, Belfast, obtained breeding pairs of S.P.F. albino rats (Wistar derived Alderley strain) from Imperial Chemical Industries in April, 1960. From these animals, employing relatively sterile techniques of handling and feeding, a S.P.F. colony of rats has been established. Sections of lungs from the animals in the S.P.F. colony at various ages are shown in Figures 2, 3 and 4. They are mounted together with similar sections from the diseased animals for comparison.

It is seen that whereas the lungs remain healthy in the S.P.F. animals, in the diseased animals, there is a steady progression of the disease process, although even at eighteen months of age, the bronchial disease seems moderate by human standards.

AIM OF PRESENT STUDY

It would seem, therefore, that as diseased and S.P.F. colonies of rats of the same strain can be established, then the S.P.F. animals could serve as normal controls and a comparative study of the two colonies may yield much information in the relationship between respiratory function and structure. Since the endemic disease in the rats histologically resembles that of human bronchitis, any experimental conclusions may possibly be applicable in the human disease. However, like the human disease, endemic respiratory disease in the rat, although predominantly bronchial, is complicated by some degree of emphysema (Fig.1), so that the separate functional evaluation of these two conditions is still not possible.

The establishment of a S.P.F. colony of rats has in fact much wider implications in research in bronchitis and other pulmonary diseases than is indicated in the above paragraph. With the elimination of endemic respiratory disease, which had tended to complicate the histological picture, the acute effects of irritant gases or the role of atmospheric pollution in inducing bronchial disease could be better studied. The pathogenesis of the industrial dust diseases and the relationship of certain of these pneumonoconioses to bronchial carcinoma and pleural mesothelioma could be more precisely determined. Besides, there is always the possibility that PPLO may play a part in the aetiology of human bronchitis,

and here knowledge of endemic rat pulmonary disease may prove an asset in future work on bronchitis.

For the present study, the determination of respiratory function in the rat is the first requisite. Reports in the literature on the study of respiratory function in small animals are scanty. Guyton (1947) described methods for measuring respiratory rate, tidal volume and minute volume in small animals, but it was felt that perhaps these were not the best parameters for detecting disturbances in function. Agostoni, Thimm and Fenn (1959) made a comparative study of the mechanics of breathing in several species, but they presented few results on the rat. Therefore, the aims of the present study are:-

(1) To develop methods of measurement of respiratory functions in the rat.

(2) To establish the normal range for a S.P.F. colony of rats with respect to age, sex and weight.

(3) To make a direct comparison of the respiratory functions between a S.P.F. and a diseased colony of rats.

(4) To correlate structural changes in the lungs of the diseased animals with their functional disturbances.

In so far as no single pulmonary function test will describe adequately the various aspects of the com-

plex process of respiration, it was decided that a number of function tests, which are most likely to show changes in the presence of bronchitis, should be performed. However, certain ventilatory tests e.g., forced vital capacity, maximum voluntary ventilation etc., are obviously impossible to do on the rat; and others e.g., alveolar air composition, diffusion capacity etc., are probably technically too difficult because of the very small volumes of gas involved. With the choice considerably narrowed, it was thought that the three most informative tests are perhaps:-

(1) The estimation of arterial blood gases.

(2) The measurement of a lung volume - the functional residual capacity.

(3) The determination of the mechanical properties of lungs.

A R T E R I A L B L O O D

G A S S T U D I E S

ARTERIAL BLOOD GAS STUDIES

In as far as pulmonary ventilation has its ultimate function in the maintenance of normal pressures of oxygen and carbon dioxide in the alveolar gas and pulmonary capillary blood, it is logical to examine the arterial blood to see how far this aim is accomplished. Using the principle that capillary blood obtained from a warm, vasodilated extremity is representative of arterial blood (Lilienthal and Riley,1944,1946), blood samples obtained from the hyperaemized tail of the rat were examined for their oxygen and carbon dioxide contents.

Method of collection of capillary blood.

Rats were warmed in a heated chamber at 37°C for about fifteen minutes. They were then placed in a tubular perspex body container which mainly served to impose some restriction on the movement of the animals (Fig.5). The tip of the tail was cut and an inflatable cuff applied onto the tail about two inches from the tip. The cuff was inflated to a pressure above the systolic pressure of the animal (usually above 120mm. Hg.) and the portion of the tail distal to the cuff was emptied of blood by a gentle milking action. The pressure in the cuff was then slowly reduced until a bright red flush spread into the distal portion of the tail. This indicated that the cuff pressure was just below the systolic blood pressure of the animal and at this point,

blood began to flow from the cut tail. The cuff pressure was maintained at this level and, after discarding the first few drops of blood, samples of 0.04 ml. were taken for analysis.

Method of analysis of blood samples.

The oxygen and carbon dioxide contents of the blood were estimated by the micro-syringe method of Roughton and Scholander (1943). For the determination of oxygen capacity, about 0.1 ml. of tail blood was collected in a watchglass and by tilting movements blood was exposed as a thin film to air. The exposure time was limited to 30 secs. (see below), after which the amount of oxygen carried in this blood was estimated as for oxygen content.

Discussion on the method used.

In trying to obtain arterialised capillary blood from a resting unanaesthetized rat, it was thought at first that local hyperaemia induced in the tail by either immersion of the tail in hand-hot water or rubbing with some histamine cream would suffice. However, these methods were found to be rather ineffective in inducing bleeding from the tail, and the whole body warming method in a heated chamber proved much more satisfactory. Excessive warming may induce hyperventilation resulting in increased elimination of carbon dioxide and respiratory alkalosis thereby altering the arterial oxygen and carbon dioxide contents (Hill and Flack,1909,

Gordon, Darling and Shea, 1949). It may also increase the body temperature and produce a shift to the right of the oxygen dissociation curve thus changing the oxygen pressure-oxygen saturation relationship (Barcroft and King, 1909). However, in practice, the period of warming was brief and, whilst peripheral vasodilatation was produced, it was unlikely that there was any great alteration in ventilation and body temperature. Furthermore, after warming, the animal was placed in a body container and allowed to settle into a resting state before blood samples were taken.

The presence of an inflated cuff kept at a pressure just below that of the systolic blood pressure, prevented the back-flow of venous blood into the distal portion of the tail during sampling. Blood samples were directly sucked into narrow calibrated pipettes and exposure to air during this manoeuvre was minimal.

In oxygen capacity determinations, as the quantity of blood available was very small, drying of the sample became a major problem and it was not possible to saturate the blood with air by the standard method described in Peters and Van Slyke (1932). Limiting the exposure time to air to 30 secs. reduced the risk of drying to negligible levels although complete saturation of the blood could not occur in that time. This would give slightly low values for the oxygen capacity, but as the effect of drying would be difficult to assess, it was decided to accept this slight limitation on the

method. Recollecting the exposed blood from the watch glass would introduce a drainage error giving values usually calculated to be too high by 0.2-0.3 vols. % (Roughton, Darling and Root, 1944). Since this would tend to compensate for the above limitation in exposure time, the error in oxygen capacity determinations was probably small.

Calculation of oxygen saturation.

In calculating the oxygen results in the syringe method, blank estimates using the same volume of distilled water instead of blood were carried out. The blank reading represents the amount of oxygen in solution in the distilled water and the reagents, and this value has to be subtracted from the oxygen readings. Assuming the amount of oxygen in solution to be the same for water and blood at the same oxygen pressure obviates the need for any correction on the oxygen capacity results. However, as arterial blood has an oxygen pressure of about 100 mm. Hg. (approximately 0.3 vols. % of oxygen in solution), whereas the same volume of distilled water used in blank estimations is at the atmospheric oxygen pressure of about 155 mm. Hg. (approximately 0.5 vols. % of oxygen in solution), subtraction of the blank reading would result in underestimation of the oxygen content by about 0.2 vols. %. Therefore in computing the final results, 0.2 vols. % was added to all oxygen content values. The oxygen saturation was then calculated as follows:-

$$\text{Oxygen Saturation} = \frac{\text{Corrected Oxygen Content}}{\text{Oxygen Capacity}} \times 100.$$

Syringe and Van Slyke methods compared.

As a check on the accuracy of the syringe method, human blood samples were analysed for oxygen and carbon dioxide contents by both the standard Van Slyke (Van Slyke and Neill, 1924) and the syringe methods. Each blood sample was analysed in duplicate by both methods and the mean reading from each method obtained. The results obtained in such a comparison are shown in Table 1. Statistical analysis indicate there is no significant difference in the methods of measurement for either oxygen ($P>0.5$) or carbon dioxide ($P>0.3$) contents.

Estimation of Blood gases in rats.

In applying the syringe method to rats, it was decided that all analyses should be made in duplicate and the mean taken as the result. With the method of sampling used, duplicate analyses in fact meant analysis of two consecutive samples of blood from the same animal. The variation of the duplicate readings is therefore expected to be greater than similar duplicate readings from the same sample. In the present study, oxygen readings were required to agree to within 0.5 vols. %, and carbon dioxide readings, to within 2 vols. %.

An initial small pilot study was carried out on rats from the S.P.F. colony, the results suggest that

the normal resting rat has an oxygen saturation ranging from 85.2% to 94.5% (Table 2). This seemed a surprised finding and further investigations into the validity of the assumption that tail samples taken in the manner described were representative of arterial blood, were undertaken.

Use of other techniques in estimating arterial oxygen saturation in the rat.

Direct left ventricular puncture was successfully performed in two animals. The results are shown in Table 2. Owing to the filling of the relatively large dead space of the syringe with heparin, the samples were well diluted. However, the oxygen saturation obtained was of the same order as the pilot study. As this procedure was done in a struggling animal, the results could hardly be compared with those obtained in the resting state.

Oximetry on shaved skin folds of the rat was next attempted. There were certain technical difficulties in doing this in the unanaesthetized animal, but the main drawback of this method was that it presupposes full saturation on breathing oxygen. Results obtained on two animals again indicate a similar degree of saturation (Table 2).

Cannulation of the aorta. It was felt that only a comparison of tail blood with an arterial sample from the same animal could prove whether tail samples

were representative of arterial blood. Permanent cannulation of the abdominal aorta in rats has been described by Still, Pradhan and Whitcomb (1956). This method was attempted, but it was found that blood flow in the tail was impaired by this procedure and tail samples were no longer obtainable. Cannulation of the aorta was next attempted through the right carotid artery using the technique described by Popovic and Popovic (1960). Under anaesthesia, a fine polythene cannula was introduced through the right carotid artery into the arch of the aorta and tied in position. The cannula was exteriorized at a point on the back of the neck to prevent interference by the animal. Rats were allowed at least five days to recover from the operation. Samples of blood from the carotid cannula and the tail could then be obtained from the same animal for gas analysis. Results obtained in such a study are shown in Table 3. Statistical analysis showed no significant difference between tail and carotid blood samples ($P > 0.5$), which confirms that tail blood samples taken in the manner described are no different from arterial blood.

Studies of arterial oxygen saturations and carbon dioxide contents in normal (S.P.F.) rats.

The oxygen content, oxygen capacity and carbon dioxide content in thirty five normal rats of either sex and different ages were determined using tail blood samples. The results are shown in Table 4 and a histogram of the data (Fig. 6) shows normal distribution. As pre-

liminary comparison showed no significant difference in oxygen saturations and carbon dioxide contents between males and females, no sex discimination was made, and all thirty five results were used in the calculation of the mean and standard deviation. Thus, oxygen saturation has a mean of 89.3% (S.D.= 2.9) and carbon dioxide content has a mean of 46.6 vols. % (S.D.= 3.3). Whilst the carbon dioxide contents are comparable in magnitude to those of the human, the oxygen saturations are below the normal range for man (93.5 - 97.5%, Douglas and Edholm, 1949). The normal resting rat is therefore, by human standards, desaturated.

Interpretation of results.

There are various possible explanations for the arterial desaturation in rats. It may be suggested that as these animals are cage-bound all their lives, the relative lack of exercise could lead to a degree of hypoventilation. This explanation seems unlikely because hypoventilation would give rise to hypercapnia as well as oxygen desaturation, whereas in fact the carbon dioxide contents are not elavated. Again, it is known that in the human, there is a small but definite alveolar air to arterial blood oxygen pressure gradient as a result of two main factors. First, a 'membrane' component due to the tissue-fluid barrier between alveolar air and the haemoglobin molecule; and second, a 'venous admixture' component due to small pulmonary venous to arterial shunts

(true shunt) and alveoli with unequal ventilation/perfusion ratios (apparent shunt) (Lilienthal, Riley, Proemmel and Franke, 1946). In resting man breathing air, the component due to unequal ventilation/perfusion ratios constitutes the largest part of the alveolar-arterial oxygen gradient (Briscoe, 1959). However, an increase in magnitude of any component will lead to a greater alveolar-arterial oxygen gradient and arterial hypoxaemia. In an attempt to find the responsible component for the arterial hypoxaemia in rats, a small group of animals were given oxygen to breath for a period of about ten minutes. Towards the end of this period, tail blood samples were taken and the oxygen contents estimated in the usual way. Oxygen contents before oxygen breathing, and, oxygen capacities were also estimated in these same animals. The results of this experiment are shown in Table 5. If it is assumed that alveolar oxygen pressure reaches 600 mm. Hg. on breathing oxygen, and the solubility of oxygen in rat blood is about the same as in human blood, then the amount of oxygen in solution will be approximately 1.8 vols.%. When the results have been corrected for dissolved oxygen, it can be seen that the rat does not reach full saturation on breathing oxygen.

This suggests that the likely cause of arterial desaturation in rats is an increase in the pulmonary venous to arterial shunt, because uneven ventilation and diffusion difficulties as causes of arterial anoxaemia are correctable by high alveolar oxygen tensions (Comroe,

Forster, Dubois, Briscoe and Carlsen, 1962).

It is also interesting to note that the oxygen dissociation curve for the rat (Jones, Maegraith and Sculthorpe, 1950) is appreciably different from that of the human (see Fig. 7) so that the upper almost horizontal portion of the curve at high oxygen pressures is absent in the rat. This means that for a given fall in oxygen pressure, there is a much greater fall in oxygen saturation in the rat, On the other hand, an arterial oxygen saturation of 90% in the rat is associated with a higher oxygen pressure (about 77 mm. Hg.) than would be the case in man. Comparison of the steep lower portion of the oxygen dissociation curves show that there is a marked shift to the right in the case of the rat. The advantage of this reduced affinity for oxygen is that more oxygen is supplied to the tissues at low oxygen pressures (Keys, Hall and Barrón, 1936). In fact, if oxygen tension in the mixed venous blood in the rat were similar in magnitude to the human, then it could be deduced from the oxygen dissociation curve that about 50% of the oxygen carried by the rat's blood must have been delivered to the tissues. In comparison, human blood under similar conditions would have given up only about 20% of its oxygen content (oxygen saturation in the human at a tension of 40 mm. Hg. is about 75%).

Comparison of arterial blood gases between S.P.F. and diseased rats.

Having defined the range of oxygen saturation and carbon dioxide content in a S.P.F. colony, a comparison of these same estimates with a diseased colony is now possible. As it has been shown that the respiratory disease progresses with age, differences between the two colonies are expected to be more apparent at older age groups. However, comparison of young animals at an age when the bronchial disease is still negligible would serve as a control. In addition, comparing animals at different age groups from the same colony may indicate the effect of aging on the blood gases.

Accordingly, arterial blood gases were estimated in twelve six-month old rats consisting of equal numbers of males and females from the S.P.F. colony. The results were compared with those obtained from the same number of rats, matched for age and sex, from the diseased colony (Table 6). The mean oxygen saturation was 90% in the S.P.F. colony and 86.7% in the diseased colony. Statistical analysis showed a significant difference in the oxygen saturation between the two groups ($P < 0.02$). There was, however, no carbon dioxide content difference ($P > 0.4$). Similar comparisons were carried out on eighteen-month old rats, there was again a difference between the oxygen saturations (89.7% v 85.9%). A comparison between the six-month and eighteen-month rats within each colony showed no significant difference.

DISCUSSION

The arterial blood gas studies suggest that there is a difference in the arterial oxygen saturation between the S.P.F. and the diseased colonies even at an age when the respiratory disease in the affected colony is histologically minimal. Because of the normal large pulmonary reserve, functional disturbances are not expected until pulmonary disease is marked. Therefore, it is unlikely that such minimal disease is the cause of an increase in arterial desaturation in the diseased colony. Furthermore, if the disease were the cause of the increased desaturation, then this desaturation should increase still further with progression of the disease; whereas comparison of the oxygen saturation between the six-month and the eighteen-month rats within the diseased colony showed no difference. If the hypothesis that arterial desaturation in the normal rat is due to pulmonary venous admixture were true, then the variation in the amount of this venous admixture in the two colonies would explain the difference in their arterial oxygen saturations.

In the human, oxygen saturation tends not to fall appreciably even when there is sufficient pulmonary disease to cause a definite fall in the arterial oxygen tension. This is because of the unique shape of the oxygen dissociation curve, so that oxygen saturation measurement is a rather insensitive index of pulmonary disease. In the rat, with the increased slope of the right hand side of the dissociation curve, it might be expected

that smaller changes in oxygen tension would be sufficient to produce detectable changes in oxygen saturation. However, it must be concluded from the present experiments that even with considerable bronchial disease in the rat, no oxygen saturation changes were detected. Recently, a micromethod for the direct estimation of blood gas tensions has been described (Bates and Oliver, 1962). Perhaps future studies using blood gas tensions may produce more informative results.

L U N G V O L U M E

S T U D I E S

LUNG VOLUME STUDIES

Lung volume determinations are anatomical measurements and do not, as such, evaluate function. However, since pulmonary disease in man is often associated with changes in the lung volume and its subdivisions, the measurement of these are of diagnostic value. Furthermore, lung volume data are used in the estimation of respiratory surface area and the number of alveoli in the lungs. Hence, lung volumes are of physiological interest and their measurement formed one of the earliest studies in pulmonary physiology.

With the invention of the spirometer by Hutchinson in 1846, the various subdivisions of the lung volume became easily and accurately measurable. However, the residual volume - the volume of gas remaining in the lungs after maximal expiration - is not amenable to direct measurement and must be determined by indirect means.

The concept of residual volume was first introduced by Davy in 1800 and he attempted its measurement using a hydrogen dilution technique (Christie, 1932). Numerous methods based on the principle of gas dilution have since been developed for the estimation of residual volume. Christie (1932) presented a comprehensive review of the various methods and suggested that as the maximal expiratory level is inconstant, measurement of the functional residual capacity (F.R.C.) is preferable to the residual volume. Gilson and Hugh-Jones (1949), after a

critical evaluation of the nitrogen wash out and helium dilution techniques for the estimation of the function residual capacity, concluded that there is accurate agreement between the two methods. However, gas dilution methods give accurate volume estimations only if all the gas in the lung is in free communication with the airways. In cases where some areas of the lung are poorly ventilated or totally obstructed, incomplete mixing of the gases occur and the estimated functional residual capacity would be smaller than the true value. To overcome this difficulty, Dubois, Botelho and Comroe (1956) recently introduced the body plethysmographic technique. This method measures the total volume of gas in the thorax whether or not in communication with the airways.

Measurement of lung volume in the rat.

Preliminary studies on lung volume in rats by Singh and Wade (1961) have indicated that lung volume is proportional to the body weight of the animal. However, their estimations were made on dead animals, the respiratory level at death was not known with certainty and it was difficult to ascertain which of the conventional subdivisions of the lung volume did the measured value represent.

It would be difficult to define and measure the subdivisions of the lung volume in the rat. But as the resting end expiratory level is usually a relatively constant point, the functional residual capacity is subject to less variation and is probably the most suitable

volume to estimate.

It was originally intended to measure the lung volume in the rat using unanaesthetized animals. But it was found that the animals tended to struggle violently when too much restraint was exercised and face masks were almost impossible to be made gas tight. What was more, the respirations of the animal became so erratic under these circumstances that even if a lung volume could be determined, it would be difficult to know which conventional subdivision of the lung volume this represented. Hence, anaesthetized and tracheostomized animals were used for functional residual capacity estimations. Tracheostomy, by excluding the dead space of the upper respiratory passages, would alter the functional residual capacity. But when all the animals were similarly prepared, it is valid to compare the results of the 'functional residual capacity' obtained.

Method of measurement of functional residual capacity.

A nitrogen dilution technique was employed. The apparatus used is shown in Figure 8. Rats were anaesthetized by intraperitoneal sodium pentothal and all measurements were made with the rat in the supine position. For ease of attachment of the tracheotomized animal to the measuring apparatus, a polythene tubing had been introduced into the tracheostomy and tied in position. The volume of the tubing was about 0.04 ml. The volume of the calibrated chamber was adjusted by altering the height of the reservoir so that it is approximately four to six

syringe the dead space of which had previously been filled with water. The initial length of the bubble was measured, oxygen was then absorbed with alkaline pyrogallol, and the final length of the bubble determined. The ratio final length / initial length x 100 then gave the nitrogen percentage in the equilibrated gas mixture.

Calculation of the functional residual capacity.

Assuming the initial concentration of nitrogen in the lung was 80%, the functional residual capacity would then be given by the following expression:-

$$\text{Functional Residual Capacity} = \frac{Va}{80 - a}$$

where 'V' is the starting volume of the chamber, and 'a' is the nitrogen percentage in the equilibrated gas mixture.

As the calibrated chamber could not contain 100% oxygen at the start of the experiment because of traces of impurities in the oxygen supply and traces of nitrogen left in the chamber when filling with oxygen, a correction factor must be applied. This factor was obtained by doing estimations of gas samples from the chamber after it had supposedly been filled with oxygen. The expression, allowing for the correction factor, would then be:-

$$\text{Functional Residual Capacity} = \frac{V(a - c)}{80 - a}$$

where 'c' is the correction factor.

The above expression had not taken into account the volume of the tracheostomy tubing (0.04 ml.). However, since this volume only amounted to about 1% of the functional residual capacity and, in any case, it tended to compensate for the reduction in respiratory dead space caused by the tracheostomy, no correction for it had been attempted.

Analysis of each equilibrated gas sample was done in triplicate; the maximal difference of the nitrogen percentages thus obtained was not more than 1% and usually less. The mean of the three readings was taken to indicate the nitrogen percentage of the gas mixture and was used for calculating the functional residual capacity. Two such equilibrated gas samples were analysed for each rat. The two calculated functional residual capacities were required to be within 10% of each other and the mean of these two values was then taken as the functional residual capacity of the animal.

Accuracy of the method used.

In an attempt to test the accuracy of the above method, syringes containing different volumes of air were attached to the chamber and, by moving the plunger to and fro, mixing of the gases were achieved. The nitrogen percentage in the chamber was determined and the volumes of air in the syringes could be calculated. Repeated estimations showed that the maximum deviation from the true volume of the syringe was 0.15 ml. and this value was inde-

pendent of the volume of the syringe used. As the range of functional residual capacity in rats was about 3-6 ml., this represented a maximum error of 2.5-5%.

Errors of the method used.

In the determination of the functional residual capacity in the rat, the method assumed that the end expiratory point at the commencement and end of the experiment was the same. Whether this was true was not known for certain, but as the end expiratory level depends largely on the mechanical properties of the lung and thorax, it is expected to be a relatively constant point. Again, the method used required the addition of oxygen to the chamber during the period of gas equilibration. Traces of impurities in the oxygen supply would have been introduced and would not have been taken into account by the correction factor mentioned above. However, because of the high degree of purity of oxygen supplies, this error is expected to be very small. Another source of error would be that as alveolar nitrogen pressure fell with oxygen breathing, the nitrogen in solution in blood and tissues of the rat would be washed out. This would cause an erroneously high nitrogen percentage in the final gas mixture. However, because of the low solubility coefficient of nitrogen, errors due to nitrogen excretion in closed-circuit methods were estimated to be very small (Christie, 1932, Gilson and Hugh-Jones, 1949).

RESULTS AND DISCUSSION

In all, the functional residual capacity of sixty nine animals were determined. The results are shown in Table 7. Analysis of the results showed that in both the S.P.F. and the diseased colonies there was a highly significant degree of correlation between the functional residual capacity and the body weight of the animals ($r = 0.8$, $P < 0.001$; $r = 0.7$, $P < 0.001$). In Figure 9, functional residual capacity is plotted against body weight to show linear relationship. Although the calculated regression coefficients for the two colonies were different, statistical analysis showed that the difference was not significant ($P > 0.3$) and, in fact, the regression lines, when plotted together (Fig. 10), showed close approximation. In contrast, age correlates poorly with functional residual capacity. It would appear that in the rat, the functional residual capacity increases rapidly with body weight up to the age of six months, then more slowly till the age of twelve months and remains steady thereafter. (Fig. 11). In the present study, there is no tendency for the functional residual capacity in the rat to increase with age as is so clearly the case in man (Frank, Mead and Ferris, 1957). As the life span of the rat is probably three years and the oldest animals studied are only eighteen months of age, it is perhaps that no sufficiently old animal has been studied to demonstrate this effect.

An increase in the functional residual capacity is associated with increase in the elastic forces which are then available for both holding the bronchi patent and increasing the energy available for expiration. It therefore offers considerable mechanical advantage in cases where there is an increase in the resistance to air flow. As the endemic disease in rats is associated with bronchial thickening, increase in airway resistance and functional residual capacity changes are to be expected. However, the results suggest that animals of the same weight from either colony would have about the same functional residual capacities. Comparing the S.P.F. and diseased animals at the same age showed that the functional residual capacities of the diseased animals are smaller because diseased animals weigh less at the same age.

As gas dilution techniques do not estimate the volume of gas not in free communication with the airways, it is possible that the method used underestimates the lung volume in the diseased rats due to the existence of poorly ventilated areas. On the other hand, like other pulmonary functions, the functional residual capacity probably does not change appreciably until the disease is marked and it is more likely that the degree of bronchial disease in the animals has not been sufficient to cause detectable changes in the functional residual capacities.

M E C H A N I C A L P R O P E R T I E S

O F L U N G S

MECHANICAL PROPERTIES OF LUNGS

Normal alveolar ventilation depends on the ability to move adequate volumes of air in and out of the lungs. Gas flow in the respiratory passages is governed by physical laws and the energy requirement for respiration is dependent on the mechanical properties of the lung-thorax system. Alteration of the mechanics of breathing by increasing the work of breathing, is perhaps the most important immediate cause of ventilatory pulmonary disability. For this reason, the mechanics of respiration particularly in man has been the subject of intensive study in recent years. Important advances has been made and a brief review of the subject is appropriate as an introduction to the studies that have been carried out in the rat.

Forces involved in the motion of the respiratory system.

In the respiratory system where the motion is cyclical changes in volume, the opposing forces may be analysed under three heads: i.e., forces which act to oppose (1) volume change, (2) volume rate of change or volume flow, (3) volume acceleration. These opposing forces, which may be expressed in terms of pressures, may be further subdivided into (a) those offered by the lungs, and, (b) those offered by the thorax.

For the lungs, the forces opposing volume change are a measure of its distensibility or compliance. The

magnitude of these forces are related only to the degree of volume change and not to the speed with which the volume change is brought about. Therefore pulmonary compliance expresses the static volume-pressure relationship of the lung. It is defined as the volume change per unit pressure change.

The forces opposing the volume rate of change of the lungs represent the frictional resistances which must be overcome in order to produce air flow. They are, therefore, a measure of pulmonary flow resistance and express the dynamic pressure-flow relationship of the lungs. Resistance is defined as the driving pressure per unit flow.

Forces related to volume acceleration of the lungs represent pulmonary inertia. The magnitude of this pressure has been estimated by Mead (1956) to be about 0.02 cm. H_2O or about 0.5% of the total transpulmonary pressure during quiet breathing in normal human subjects. Although this pressure increases with more rapid volume accelerations, it forms such a small proportion of the total pressure that for all practical purposes it may be regarded as negligible.

PULMONARY COMPLIANCE

Surface tension and volume-pressure behaviour of the lungs.

Donders (1849, 1853) first described the retractive properties of lungs and showed that the retractive

force increased as the lungs were inflated. This was also noted by Hutchinson (1852) on studying the volume-pressure relationships on two human lungs immediately post-mortem. Earlier workers attributed pulmonary retraction to the elastic properties of the lung tissue but Neergaard (1929), by comparing volume-pressure curves of air-filled and liquid-filled lungs, demonstrated that surface tension was responsible for a large proportion of the total retractive force. However, it was a strange coincidence that the early workers, in their study of volume-pressure relationships of lungs, used either inflation or deflation curves so that the phenomenon of pulmonary hysteresis was not appreciated until the more recent studies of McIlroy (1952) and Radford (1957). Hysteresis has been defined by Landowne and Stacy (1957) as the failure of a system to follow identical paths of response upon application of and withdrawal of a forcing agent. The result of this failure to retrace the same path on withdrawal as on application is the formation of an hysteresis loop.

Air inflation and deflation of lungs from the collapsed state exhibited marked hysteresis. However, Mead, Whittenberger and Radford (1957) found that when the surface forces were minimized by liquid-filling of the lungs, the volume-pressure curve then showed comparatively little hysteresis. They concluded that surface phenomenon was responsible for most of the observed hysteresis.

The discovery by Pattle (1955, 1958) that pul-

monary oedema fluid formed stable bubbles and exhibited very low surface tensions stimulated further investigations into the role of surface forces. Brown (1957) and Clements (1957) using different methods demonstrated that lungs and films of lung extracts expand with a high surface tension but on compression the surface tension falls off rapidly to low values.

Two mechanisms have been postulated in which surface tension might contribute to pulmonary hysteresis. Mead, Whittenberger and Radford (1957) suggested the following explanation. During inflation from the gas free state, the total number of air spaces sharing the volume increases progressively. Volume increase of the lungs is partly accompanied by further expansion of air spaces already open and partly by recruitment of new air spaces. During deflation the air spaces remain open over much of the volume range. In this way, a given volume is shared by a larger number of air spaces at any volume during deflation than during inflation. The larger the number of air spaces sharing the volume, the smaller the pressure developed by the lungs. All pressures during inflation would then exceed those at equal volumes during deflation and a volume-pressure cycle would exhibit hysteresis. The observation that air-filling of lungs from the gas-free state is irregular and non-uniform (Lawton and Joslin, 1951, Radford, 1957) lends support to the possibility of recruitment of alveoli during inflation. Bernstein (1960), in studying volume-pressure curves in

rabbit lungs, also proposed the existence of alveolar recruitment.

The second mechanism is due to the surface tension-area hysteresis described by Brown, Johnson and Clements (1959). If the individual air spaces inflate and deflate with different surface tensions, then the pressures during inflation would exceed those at equal volumes during deflation and volume-pressure hysteresis is to be expected. Furthermore, Clements, Brown and Johnson (1958) have pointed out that area-surface tension hysteresis would also contribute to the first mechanism to the extent that it extends the range of stable behaviour of air spaces during deflation.

Tissue forces and volume-pressure relationships of the lungs.

Although smaller in magnitude than the surface forces, pulmonary tissue contributes to the total retractive force of the lungs. In studies in liquid-filled lungs where surface forces are minimized, pressure increases progressively with volume showing a greater increase at high lung volumes (Radford, 1957). Setnikar and Meschia (1953) have pointed out that if pulmonary tissue lengthens approximately as the cube root of volume, then linear elasticity of the tissue elements would not result in linearity of volume and pressure. For pressure to increase progressively with volume, increase in tension in the tissue must be proportionately more than its increase in length. These observations were confirmed by length-tension studies on lung tissue slices from dogs by Radford

(1957). Furthermore, Mead, Whittenberger and Radford (1957) have shown that volume-pressure curves predicted from length-tension data closely resembled those obtained from liquid-filled lungs.

The stability of air spaces.

The functional significance of the surface tension and tissue characteristics is probably in the maintenance of stability of the air spaces. Pulmonary air spaces are essentially single-surfaced bubbles contained within distensible elastic sacs. When numerous such structures are connected in parallel as is the case in the lung, both the surface and the tissue become potential sources of instability. The behaviour of a soap bubble blown on a tube has been cited as an example of surface instability (Neergaard, 1929, Mead, 1961). Here, the pressure difference (P) across the spherical surface of the bubble is expressed by La Place's equation:-

$$P = \frac{2T}{r}$$

where 'T' is the tension in the surface (surface tension), and 'r' is the radius of curvature of the bubble.

As the bubble forms 'r' will decrease to a minimum value, when the bubble radius equals the radius of the tube, and then increase again. If surface tension remains unchanged then the pressure will have a maximum corresponding to the minimum radius of the bubble. If the above volume-pressure relationship were represented gra-

phically, then it will be seen that initially volume increases with pressure until the bubble reaches the hemispherical shape ('r' reaches its minimum value), when further increase in volume will result in decrease in pressure. Where the slope of the curve is positive, the bubble is stable because volume changes induced by changes in applied pressure are such as to produce equal and opposing pressures. Where the slope is negative, changes in applied pressure produce inappropriate changes in opposing pressures and the bubble either bursts or collapses to its lower stable volume.

In the lungs, there is evidence that the alveoli probably exceed the hemispherical stage. During air inflation the sudden filling of structures of alveolar dimensions as observed by Mead, Radford, Ferris and Whittenberger (1955) suggests these spaces are passing through unstable states. If this were so, then stability could only be maintained by tissue elements whose length-tension characteristics would prevent hyper-expansion and confer stability to the air spaces during inflation. During deflation the sharp fall-off of surface tension would extend to lower volumes the range of stable behaviour of air spaces. A degree of stability of the air spaces is thus achieved during both phases of the respiratory cycle.

If alveoli exceeded the hemispherical shape and stability is conferred by tissue restraint then the system exhibits bi-stable behaviour. That is, there is an initial stable region until the hemispherical shape

is reached, beyond this is an unstable region and finally an upper stable region. A system consisting of such bi-stable units will itself show hysteresis because on inflation there are fewer units in the upper stable region than at comparable applied pressures on deflation (Mead, 1962). This is then another possible mechanism in the volume-pressure hysteresis of the lung.

The practical evaluation of pulmonary compliance.

The retractive characteristics of the surface and tissue have been discussed in detail because these are in effect the properties measured when determining pulmonary compliance. The complex nature of the retractive forces makes it difficult to describe them adequately by any simple mathematical expression. However, marked static hysteresis occurs only when lungs are inflated from the collapsed state whereas normal breathing with small volume cycles will occupy only a small portion of this curve. Butler, White and Arnott (1957) have shown that in the range of spontaneous breathing static hysteresis is much reduced. Furthermore, volume-pressure relations in the normal tidal range is almost linear. It is thus possible to express pulmonary compliance simply by the slope of the linear portion of the curve.

However, many factors complicate the practical evaluation of pulmonary compliance. Butler et. al.(1957) emphasized that the volume-pressure relation of the lung is everywhere curved and only approximately linear in the mid range. The implication is that compliance would

change with lung volume even though it is measured in the tidal range.

The influence of lung volume on pulmonary compliance may be illustrated by the following model. If two balloons of equal volume and compliance are connected to a Y tube and if a unit change of pressure (dP) causes a change in volume dV in each balloon, then the compliance of the system is 2dV/P. Now, one balloon is blocked off from the source of pressure. The same change in pressure will then only produce a volume change of dV, that is, the compliance is halved although there has been no change in the elastic properties of the balloons. If the compliance results are related to the initial volumes of the system in each case, then the above discrepancy will not arise. Therefore pulmonary compliance measured in the tidal range will only be meaningful if evaluated in relation to the functional residual capacity.

Bernstein (1957), Mead and Collier (1959) and Ferris and Pollard (1960) observed that compliance tended to decrease with time and that this was reversible by single near-maximal inflations. Bernstein (1957) attributed this phenomenon to progressive closure of air spaces and this was supported by the observation of areas of atelectasis in the lungs of animals not receiving intermittent forced inflations by Mead and Collier (1959). Whatever the explanation, there is evidence that the volume history of the lungs played a part in determining the measured compliance.

Neergaard and Wirz (1927) introduced the concept that at instants of zero flow in the respiratory cycle, static conditions were present, the pressures at these points would then only relate to volume and pulmonary compliance could be determined. In normal lungs, the value of this dynamic compliance is similar to that obtained under static conditions. Furthermore, it has been shown by Butler, White and Arnott (1957), Mead and Whittenberger (1953) and Otis, McKerrow, Bartlett, Mead, McIlroy, Selverstone and Radford (1956) that dynamic compliance is independent of breathing frequency in normal subjects. Otis et. al. (1956) suggested that in a system of parallel pathways such as in the lungs, for dynamic compliance to be independent of cycling frequency, the mechanical time constants for the separate pathways must be the same. They predicted that if this were not so, then dynamic compliance would decrease with increase cycling frequency. The finding that dynamic compliance became frequency dependent in induced bronchospasm, in asthmatic patients (Otis et. al., 1956), and in patients with emphysema (Mead, Lindgren and Gaensler, 1955) - conditions in which the mechanical time constants might be expected to differ - lends support to their postulation.

In summary, pulmonary compliance measured in the tidal range may change with lung volume, with volume history and with breathing frequency. It is by definition the static volume-pressure relationship of the lung; it

should be measured under static conditions and whenever possible, information as to lung volume and volume history should be included. Compliance is a measure of surface and tissue properties of the lung; pleura, bronchi, blood vessels and smooth muscle probably all contribute to the tissue component (Radford, 1957). Interpretation and comparison of compliance results must take into account all these limitations.

PULMONARY RESISTANCE

Pressure-flow relationships in pulmonary resistance.

In 1915, Rohrer presented a comprehensive analysis of respiratory mechanics. By applying the laws governing gas flow through tubes to post-mortem measurements of the dimensions of the air passages, he estimated the resistance to gas flow as well as the contribution of the various parts of the airway to the total resistance. He described 'tubular' and 'additional' flow resistances and calculated incorrectly that all tubular flow would be laminar in character even during rapid breathing. Thus according to the Hagan-Poiseuille Law, tubular flow-resistive pressures would be directly proportional to flow, the pressures due to additional resistances would be proportional to flow squared and total flow-resistive pressure would be the sum of the two pressures i.e.,

$$\text{Total flow-resistive pressure} = K_1(\dot{V}) + K_2(\dot{V})^2$$

where $K_1(\dot{V})$ represents the pressure of laminar flow, and $K_2(\dot{V})^2$ represents the pressure of additional resistances, and $(\dot{V})$ is the volume flow.
K_1 includes the viscosity of the gas and the tubular geometry. K_2 includes the density of the gas, the cross-sectional and the bend geometry of the bronchial tree.(Mead, 1961).

The critical point at which gas flow in a tube changes from laminar to turbulent can be estimated by calculating the dimensionless Reynolds' number. Gaensler, Maloney and Björk (1952) and Mead (1961) presented calculations indicating that in the larger airways the critical Reynolds' number would easily be exceeded even during moderate hyperventilation and Rohrer's (1915) assumption that all tubular flow would be laminar was correct only for quiet breathing. For turbulent flow, pressures are approximately proportional to flow squared so that Rohrer's expression might still describe the pressure-flow relationship. Determinations of K_1 and K_2 has been undertaken by Mead and Whittenberger (1953) under conditions when changes in airway dimensions due to respiratory movements were minimal. McIlroy, Mead, Selverstone and Radford (1955) attempted to alter K_1 and K_2 by altering the viscosity and density of the respired gases. Their results indicate that K_1 and K_2 do not alter as predicted by changing the physical properties of the respired gases. The physical meaning of these constants therefore remain in doubt.

If the respiratory passages were rigid tubes of fixed dimensions, then Rohrer's expression might approximately describe their pressure-flow relationships. However, in life, the dimensions of the air passages change with lung volume, change at the same volume with rate of flow, and furthermore, the change in dimensions is opposite in direction and unequal in magnitude during inspiration and expiration. Recent work has concentrated on the measurement of flow-resistances during life, the separation of the total flow-resistive pressure into its gas and tissue components and the static and dynamic volume-pressure-flow relationships of the lungs.

Measurement of flow-resistive pressures and pulmonary flow-resistance.

It has been indicated at the beginning of this section that the pressures opposing the motion of the lungs may be analysed in terms of volume change, volume flow and volume acceleration. According to Newton's Third Law of Motion, the sum of these opposing pressures must equal to the applied pressure. If the lungs behave as a mechanical system in which volume is the only variable in determining its configuration at any time, i.e., with one degree of freedom, then the equation of motion for such a system may be written:-

$$P = F1V + F2\dot{V} + F3\ddot{V}$$

where P is the applied pressure and the opposing pressures are functions of volume (F1V), volume flow (F2$\dot{V}$)

and volume acceleration ($F_3\ddot{V}$).

If inertial pressures are negligible, then flow-resistive pressures at any point may be solved by subtracting the elastic pressures from the total transpulmonary pressure. If instantaneous flow rate is obtained at the same time, pulmonary resistance can be computed. This principle was first employed by Neergaard and Wirz (1927) in measuring pulmonary resistance, it has since been simplified by Mead and Whittenberger (1953) by the use of electrical subtraction of the compliance pressures from the total transpulmonary pressure. A limiting factor is that this method subtracts the dynamic compliance pressures and where dynamic compliance changes with breathing frequency, the meaning of the flow-resistive pressure obtained is in doubt.

Neergaard and Wirz (1927) also introduced a method for measuring alveolar pressure. They reasoned that after interruption of flow, mouth pressure would equilibrate to the pressure existing in the alveoli prior to interruption. It was thought that pulmonary gas flow resistance could then be estimated and partitioning of total flow resistance into its gas and tissue components could be accomplished. However, Mead and Whittenberger (1954) estimated that under such circumstances, alveolar pressure would have changed substantially prior to such equilibration and that the pressure measured would more nearly approximate the total flow-resistive pressure. This was supported by the finding that flow resistances

obtained by this method had similar values to the total pulmonary resistance measured by other means.

Separation of total pulmonary flow resistance into gas and tissue components.

McIlroy, Mead, Selverstone and Radford (1955) attempted the separation of gas and tissue resistances by changing the physical properties of the respired gas thus altering only gas flow resistance. By using gases of equal kinematic viscosity, the character of flow in the respiratory passages would remain substantially unchanged. They estimated that tissue resistance is approximately linear and accounts for 30-40% of the total pulmonary resistance. Dubois, Botelho and Comroe (1956) introduced the body plethysmographic method for measuring alveolar pressure. In this method the subject pants through a flow-meter and fluctuations in chamber pressure are related to alveolar pressure by closing off the flow-meter whilst the panting continues. The alveolar pressure and simultaneous flow at any point can be obtained. The calculated tissue resistance by this method is about 18% of the total (Marshall and Dubois, 1956). Both these methods require breathing patterns differing appreciably from the normal and Mead (1961) has suggested that the differences in the estimated pulmonary tissue resistance may be related to this factor.

Partitioning of airway resistance between the various air passages.

Rohrer (1915) predicted that nasal resistance would account for 47% of the total airway resistance during quiet breathing. Butler (1960) found wide variations in nasal resistance ranging from 40-80% of the total in normal people. Furthermore, it might change suddenly and inexplicably in the same individual on repeated measurements. Mead (1961) estimated from Rohrer's data that during quiet mouth breathing, the upper airways including the glottis would account for 25% of the total. This is in agreement with Hyatt and Wilcox (1960) who found values of between 19-29%. The distribution of resistance between the various pathways from trachea to the air spaces is unknown, but seems to be related to their elastic characteristics so that distribution of ventilation is independent of the breathing pattern in normal lungs (Otis et. al., 1956).

The volume-pressure-flow relationships in the lung.

(a) Airway conductance and lung volume.

Rohrer (1915) predicted that airway conductance (inverse of resistance) and airway volume would be positively correlated in most instances. Mead and Whittenberger (1953) found that pulmonary resistance increased with decreasing lung volume approximately two and a half times over the vital capacity volume range. Briscoe and Dubois (1958) made an extensive study of the relationship between lung volume and airway conductance and found a positive correlation whether the lung volume difference be

due to growth, individual variation or degree of inflation. In the case of degree of inflation, the lung volume and airway conductance were found to be nearly linearly related. If upper airway resistance is assumed to be 25% of the total and changes little with lung inflation, then the observed relation represents a disproportionate increase in conductance to lung volume. If conductance and airway volume are positively correlated then the implication is that airway volume increases out of proportion to lung volume during inflation. The finding by Martin and Proctor (1958) that radius changes is greater than length changes in dog bronchi supports this.

(b) Dynamic changes in airway resistance.

Einthoven (1892) was the first to point out the influence of intrathoracic pressure on the air passages. During inspiration, pleural pressures become more negative and there is a pressure gradient along the respiratory passages ranging from near atmospheric in the trachea to a negative pressure in the alveoli. The negative pleural pressure has a tendency to widen the airways, this being greatest in the large airways and least in the small ones. During expiration the pressure gradient is reversed, the alveoli now have positive pressures. If pleural pressure rises above atmospheric as is the case during rapid expiration, then compression of the airways occurs, again this being most marked in the larger airways where intraluminal pressures are smallest. Einthoven's predictions have been confirmed by Dekker, Defares and

Heemstra (1958) who concluded from direct measurements of intrabronchial pressures at various levels that in normal subjects, the increased expiratory resistance during rapid expiratory efforts occurred predominantly in the larger airways. There are bronchoscopic, bronchographic and cinefluoroscopic studies supporting these findings (Gandevia, 1963, Bates, 1962). However, as pointed out by Dayman (1951) the larger airways which bear the brunt of these pressures are to some extent protected by cartilage.

There is evidence that during normal breathing airway widening and compression do not occur to any appreciable extent. Mead and Whittenberger (1953) found that pulmonary flow resistance was approximately the same for inspiratory and expiratory phases for flow rates up to 2L./sec. Although with rapid expiratory efforts increase in expiratory flow resistance as much as twenty fold may occur, no comparable decrease is present during inspiration. The discrepancy between the phases can be explained on the basis that during rapid expiration, airway compression leads to increased resistance and thus further narrowing downstream, whereas during inspiration, airway widening reduces the flow-resistive pressures and hence less widening.

Dayman (1961) analysed the expiratory spirogram in detail and showed that it could be divided into two main phases. In the initial phase, rate of flow was effort dependent; this was soon followed by a phase of

critical flow during which flow could not be increased by effort once a certain threshold of pressure was exceeded. At a given point in the vital capacity, critical flow was fixed; the rate of critical flow being directly related to the degree of lung inflation. Fry and Hyatt (1960) described isovolume pressure-flow curves and found that except high in the vital capacity, flow maxima could be attained with moderate effort at any specific volume. With increased efforts, flow might actually decrease and since the applied pressure increased sharply with effort, the measured resistance would increase disproportionately. Dynamic narrowing with increase in airway resistance thus imposed a flow maxima at each volume and this flow maxima decreased with lung volume. Hyatt, Schilder and Fry (1958) suggested that the degree of dynamic airway narrowing could be expressed by plotting flow against volume during a series of moderately forced expirations from different lung volumes. Approximately the lower two-thirds of such curves then represented the maximum flow-volume relationship. This was reproducible and largely independent of effort.

(c) Pulmonary resistance in emphysema.

Dayman (1951) demonstrated very marked increases in resistance during expiration in patients with pulmonary emphysema. He postulated that normally lung retraction provided radial forces which act on the walls of the small airways and prevent dynamic narrowing. In emphysema, intrinsic narrowing of the airways, decrease in

retractive force of the lungs leading to loss of radial support of airways and a less negative pleural pressure all contribute to increase in expiratory resistance. In fact, increase in airway resistance from any cause, by increasing the pressure required to produce a given flow and by decreasing the intraluminal pressure in the airways proximal to the obstruction favours further dynamic compression of proximal airways.

In summary, pulmonary flow resistance changes with lung volume, with rate of flow, with effort and with the phase of respiration. In normal breathing, and, if extremes of flow rate and effort are avoided in rapid shallow breathing, then the difference in resistance between inspiration and expiration is small and lung volume then becomes the determining factor. Separation of total resistance into gas and tissue components has yielded different results probably because of the breathing pattern required by the methods. Measurement of flow-resistive pressure requires the subtraction of dynamic elastic pressures except in the body plethysmograph method. In diseased states when dynamic compliance is frequency dependent, the meaning of the flow resistance is restricted to the particular frequency of measurement. Measurement of airway resistance by the body plethysmograph is also complicated in these circumstances because of the existence of differences in pressure in the air spaces. Finally, in abnormal airways, previous volume history is important

to the extent that a single deep inspiration may be followed by marked temporary decrease in airway resistance (Nadel and Tierney, 1961).

STUDY OF THE MECHANICAL PROPERTIES OF LUNGS IN THE RAT.

Method

The general arrangement of apparatus is shown in Figure 12. Rats were anaesthetized by intraperitoneal sodium thiopentone and all measurements were made with the rat in the supine position.

Measurement of intrapleural pressure.

Intrapleural pressure was measured using a fluid-filled tube inserted into the pleural cavity as follows:-

A rigid needle with an internal diameter of 1 mm. was pushed through an intercostal space in the right lateral chest wall near the inferior angle of the scapula, traversed the pleural cavity, and emerged again through a lower space. A polythene tube about twelve inches long with an internal diameter of 0.5 mm. was threaded through the needle which was then withdrawn leaving the polythene tube traversing the pleural space. The whole tube was then filled with water and three side-holes were made in the tube for the transmission of pressure changes. One end of the tube was sealed and the other end was connected through a three-way tap to one side of a differential electromanometer. The other side of the manometer was open to air. A water manomater was connected to the remaining arm of the three-way tap. It was then possible to use the water manometer either for calibration of pressure changes or for flushing the

intrapleural catheter. The differential electromanometer was connected through an amplifier to a cathode ray oscilloscope which monitored the pressure changes. The oscilloscope was also connected to a direct writing four channel recorder so that a record of the pressure changes could be obtained. The position of the tube was adjusted so that the side-holes lied in the intrapleural space, this manoeuvre was achieved with the aid of the monitoring system. Records of intrapleural pressure swings were thus obtained.

The side-holes in the intrapleural catheter tended to become blocked after some time. When this occurred the pressure tracings would be reduced in amplitude and sometimes also became abnormal in shape. With a little experience, these changes were easily recognizable and the side-holes could be flushed clear again. In order to avoid flooding the intrapleural space, the side-holes were withdrawn from the pleural cavity when they were being cleared. The position of the intrapleural catheter was checked at post-mortem in every case. With experience the needle track usually traversed the pleural space without damaging the underlying lung. Occasionally, the tube may pass through a corner of the lower lobe of the right lung, but results obtained in these animals were not significantly different from the rest. Amdur and Mead (1958) using a similar technique in guinea pigs also came to this conclusion.

Measurement of volume flow and flow rates.

Respiratory flow rate was measured using a Godart pneumotachograph which also integrated the flow signals to give volume flow. Thus, both volume flow and flow rate could be recorded via the direct writing four channel recorder. The standard 0-10 L. pneumotachograph head had to be modified in order to measure the very low flow rates of the rat. By considerably narrowing its bore the dead space of the pneumotachograph head was reduced and the small tidal volumes of the rat then produced a measurable flow. However, the modification still left the bore of the measuring head many times the size of the trachea of the rat. In fact, estimation of the resistance of the head showed that no measurable resistance was encountered for flow rates up to 25 ml./ sec. Calibration of the flow rate was by means of an air displacement method. Water under a more or less constant head of pressure was allowed to flow into a graduated cylinder and the air displaced was made to flow through the measuring head. Knowing the time it took to displace a given volume, volume flow per unit time could be calculated. By varying the head of pressure, different flow rates were produced and the response of the measuring head was found to be linear within a flow range of 2-12 ml./sec. Calibration of volume flow was accomplished by moving measured amounts of air to and fro through the measuring head using a lubricated syringe.

Measurement of pulmonary compliance.

An attempt was made to measure pulmonary com-

pliance under static or near static conditions. Spontaneously breathing, anaesthetized and tracheostomized animals were used. A tap with a side-arm was connected to the tracheostomy tube so that the side-arm was on the tracheal side of the tap (Fig. 13). A fluid-filled tube leading from the side-arm was connected to one side of the differential manometer and the intrapleural catheter to the other. Changes in transpulmonary pressure were thus recorded using the direct writer. Calibration was by the same water manometer already described.

A pause at end expiration was present in the breathing pattern of anaesthetized rats. Static conditions were produced at this point by closing the tap. The animal would continue to make respiratory efforts, but end-expiratory transpulmonary pressures would be registered at the points of respiratory pauses when the animal was not making any active respiratory efforts. A series of these points would describe a straight line on the record, representing the level of transpulmonary pressure (see Fig. 14). After three to four respiratory cycles, 0.4 ml. of air was added into the system using a tuberculin syringe by momentarily opening the tap — the syringe with its 0.4 ml. being connected in readiness immediately after the tap was first closed. The increase in volume, by increasing lung retraction, produced a change in the transpulmonary pressure indicated by the displacement of the line of respiratory pauses. The magnitude of displacement then gave a measure of the change in trans-

pulmonary pressure. The time required for a single determination was about 10 secs. The process was repeated after the breathing pattern of the animal was judged to have returned to normal. The average of three readings was taken as the mean change in transpulmonary pressure produced by a volume change of 0.4 ml. Pulmonary compliance could then be expressed as the ratio: Volume change/Transpulmonary pressure change (ml./cm. H_2O).

Measurement of pulmonary resistance.

Two methods were used in connecting the rat to the pneumotachograph head. In the first, a face mask was employed. The mask was made from the cast of a rat's head, it had a wide exit. In order to ensure an air-tight fit, the rat was shaved about its face and the margins of the mask were coated with 'Aquaresin' (A glue-like substance which has the advantage of being soluble in water — from Glyco Product Co. Inc., N. Y.). This method measured the total flow resistance including the upper airways.

In the second method, the animal was tracheostomized and the tracheostomy tube connected to the measuring head through a rubber cork. In this case resistance of the glottis and upper respiratory passages had been excluded and the resistance measured would be referred to as the lower pulmonary resistance.

Method of analysis for pulmonary resistance.

The method used was that described by Amdur and

Mead (1958). The intrapleural pressure has an elastic and a flow-resistive component. At points of equal volume during the respiratory cycle, the elastic forces would be nearly equal and the pressure change between two such points would relate chiefly to resistance to flow offered by the airway and tissues. This pressure change when related to the associated flow change would give a measure of pulmonary flow resistance (cm. H_2O/ml./sec.). (see Fig. 15).

Discussion on the methods used.

Pulmonary compliance and resistance were estimated by relating changes in transpulmonary pressure to changes in volume and flow respectively. The measurement of intrapleural pressure assumed that the pressure over the surface of the lungs were uniform. Wiggers, Levy and Graham (1947) presented evidence that pressures in the region of the heart were less subatmospheric. Farhi, Otis and Proctor (1957) studied intrapleural pressures at different points in the chest of the dog and showed that although local pressure differences existed, the magnitude of this difference was small especially in the mid thoracic region. However, the possibility remained that minor local pressure differences might complicate all measurements of intrapleural pressure.

In compliance determinations, the assumption was made that the volume-pressure relationship in the rat lung was linear in the tidal range. The tidal volume in anaesthetized rats was found to be about 0.8-2.0 ml.

A volume change of 0.4 ml. was chosen because this was well within the tidal volume of all rats and still produced a measurable transpulmonary pressure change of about 2 cm. H_2O. A larger volume change or a series of volume changes would perhaps have been more preferable because the accuracy of measurement might be improved.

The objection to measuring compliance during breathing was that if the mechanical time constants in the various lung pathways differed, then ventilatory distribution and hence compliance became frequency dependent. In an attempt to overcome this difficulty, compliance was measured during airway obstruction. Respiratory efforts during obstruction would cause compression and expansion of the alveolar gas which might produce some intrapulmonary flow. But frequency dependent distributional inequalities would be largely minimized and probably non-existent at points of respiratory pauses when airway pressures everywhere would equilibrate.

The method employed also assumed no change in lung volume (other than the 0.4 ml. introduced) after airway obstruction. For an obstruction time of only 10 secs., this assumption was probably justifiable, whereas with longer obstruction times appreciable changes in volume might be expected if a low respiratory quotient were operative.

The use of tracheostomized animals could be criticized, however, there was no reason to expect that this would alter pulmonary compliance. The finding by

Amdur and Mead (1958) that compliance in guinea pigs did not change with tracheostomy would support this.

It was not unexpected that a period of deep breathing should follow airway obstruction. This would have an alveolar opening effect so that the volume history of the above method was such that pulmonary compliance was probably measured at a time when the majority of the alveoli were open.

The method of measurement of pulmonary resistance did not permit its separation into airway and tissue components. It gave the mean inspiratory and expiratory resistance near peak inspiratory and expiratory flow rates (peak flow rate in the anaesthetized rat was about 8-12 ml./sec., see Fig. 15). As had been shown by Mead and Whittenberger (1953) during quiet breathing resistance was very nearly linear and there was little difference between inspiratory and expiratory resistances. Therefore the method employed probably measured the average pulmonary resistance during the respiratory cycle. It was indicated that for methods which relied on subtraction of dynamic elastic pressures from the total in obtaining flow-resistive pressures, then frequency dependency of dynamic compliance became a limiting factor. The present method of analysis for pulmonary resistance assumed that elastic pressures at the same lung volume was approximately equal. This assumption would be valid whatever the breathing frequency because for any given frequency the dynamic compliance at equal volumes must still

be nearly the same. On the other hand, pulmonary hysteresis would produce different pressures at the same lung volume and should be considered as a potential source of error in this method.

RESULTS AND DISCUSSION

The values of pulmonary compliance and flow resistance obtained in normal and diseased rats are shown in Table 8. The normal group consists of animals of both sexes with ages ranging from six to eighteen months. In this group, the mean pulmonary compliance was 0.25 ml./cm. H_2O. This is almost one thousandth of the values obtained in the human by Butler, White and Arnott (1957), Frank, Mead, Siebens and Storey (1956) and Marshall (1957). However, if the mean functional residual capacity is 4.0 ml. in the rat and approximately 3.0 L. in the human (Bates and Christie, 1950, Whitfield, Waterhouse and Arnott, 1950), then corrected for lung volume differences, the rat and human lung would appear to be about equally compliant. Amdur and Mead (1958) obtained similar compliance values in guinea pigs but their data did not include information on the lung volumes.

In normal rats, the mean total pulmonary resistance was 0.23 cm. H_2O/ml./sec. with a standard deviation of 0.09. Since with the present method of measurement using a face mask, the animal could have breathed through its nose, its mouth or both, a wide range of results was to be expected. In guinea pigs, Amdur and Mead (1958) reported a mean total pulmonary resistance of 0.69 cm. H_2O/ml./sec. with a similarly wide range. Agostoni, Thimm and Fenn (1959) in their comparative study have indicated that the guinea pig tends to have a smaller lung volume

per unit body weight than other animals. As they are also prone to develop bronchospasm, it is not surprising that high resistance results were obtained.

The mean lower pulmonary resistance in the normal rat was 0.14 cm. H_2O/ml./sec. with a smaller standard deviation of 0.04. Thus, pulmonary resistance in the rat is approximately one hundred times the human values as given by Mead and Whittenberger (1953) and Frank, Mead and Ferris (1957), whilst its lung volume is probably nearer a thousandth. If pulmonary resistance is inversely proportional to airway volume, it is tempting to speculate from these findings that the rat should have a disproportionately larger airway volume than man. The radius of the trachea is about 1 mm. in the rat and 10 mm. in the human (Rohrer, 1915) so that the cross sectional area difference would be about a hundred fold. Although the trachea is not representative of the total airway volume, this finding is at least consistent with the above postulation.

The partitioning of airway resistance between the various respiratory passages in the human has been discussed in a previous section. Amdur and Mead (1958) found that in guinea pigs upper airway accounts for about 45% of the total flow resistance. The present small series showed that where both total and lower pulmonary resistance were measured in the same animals, the estimated upper airway resistance varied widely ranging from 41-69% with an average of 53% of the total. As cited above,

one possible explanation for this variation is related to whether mouth or nose breathing took place during the measurements.

As there is histological evidence that the bronchial disease in rats progresses with age and becomes marked after the age of twelve months, the diseased animals were arbitrarily divided into two groups for the purposes of comparison using twelve months as the dividing line.

Comparison of pulmonary compliance shows that a significant difference exists between the normal and the older diseased groups. Preliminary observations showed that the total pulmonary resistance between the normal and the young diseased groups was not different. The disadvantage of comparing total pulmonary resistance is that if upper airway resistance accounts for a large part of the total, as is probably the case, then changes in lower pulmonary resistance would only cause small changes in the total and therefore not easily detectable. Furthermore, upper airway resistance may change suddenly and unpredictably with changes in attitude of the tongue and pharynx (Butler, 1960, Fry and Hyatt, 1960). For these reasons, comparison of lower pulmonary resistance using tracheostomized animals was preferred. Statistical analysis indicates that not only is there a difference between the normal and the older diseased groups, but that the difference between the two diseased groups is also highly significant. There is no significant difference

in lower pulmonary resistance between the normal and the young diseased groups.

The influence of lung volume on the values of pulmonary compliance and resistance has been discussed in a previous section. The above comparisons between the normal and diseased animals did not allow for lung volume differences so that their validity may be doubted. Functional residual capacities have not been obtained in the same animals in which pulmonary compliance and resistance were determined, but as there is a high degree of correlation between functional residual capacity and body weight (see previous section on lung volume), it seems reasonable to correct pulmonary compliance and resistance results against weight. Compliance results are divided by body weight whereas resistance, being inversely proportional to lung volume, is multiplied by the body weight. Statistical analysis of the corrected data now showed no significant difference in pulmonary compliance between the normal and the older diseased groups whereas pulmonary resistance between these same groups remained highly significant indicating that there is an increase in lower pulmonary resistance in the endemic bronchial disease in rats.

S U M M A R Y

S U M M A R Y

There is evidence that in human bronchitis and emphysema, functional disturbances do not correlate well with structural changes. Experimental studies in animals offer the advantage that histological details of the lungs can be made available immediately after respiratory function studies have been carried out. Laboratory rats suffer from an endemic respiratory disease which is histologically similar to human bronchitis. It has recently become possible to obtain rats free from respiratory disease and a colony of such rats has been established. It was hoped that a comparative study of a healthy and diseased colony of rats might yield some information on the relationship between pulmonary structure and function.

There have been few previous attempts to study respiratory function in small animals. There is no respiratory functional data on rats known to be free from respiratory disease. This thesis reports the development of three tests of respiratory function in the rat:

(1) Arterial blood gas studies,

(2) Lung volume determinations,

(3) Estimation of the mechanical properties of the lungs,

and the result of a comparative study of these functions in healthy and diseased rats.

In normal unanaesthetized rats, the mean arte-

rial carbon dioxide and oxygen contents were 46.6 vols.% and 19.1 vols.% respectively. The mean oxygen capacity was 21.4 vols.% giving a mean oxygen saturation of 89.3%. The arterial desaturation was not completely corrected by oxygen breathing and it was suggested that venous admixture was the probable underlying cause. Since the blood oxygen dissociation curve is shifted markedly to the right in the rat, the arterial desaturation is probably not associated with marked reductions in oxygen tension as would be the case in man.

Functional residual capacities were estimated in rats of both sexes with ages varying from two to eighteen months. The range of volumes obtained was 2-6 ml. There was a high degree of correlation between functional residual capacity and body weight.

Pulmonary compliance was determined under static conditions. The mean value obtained was 0.25 ml./cm. H_2O. Pulmonary resistance measurements showed a wide range of variation explicable on the basis that the method used permitted the rat to breath either through the nose or mouth. In tracheostomized animals, pulmonary resistance was found to be 0.14 cm. H_2O/ml./sec. The upper airways including the glottis accounted for 53% of the total resistance.

The diseased animals showed a significant increase in arterial desaturation. Since this occurred in young animals in which the bronchial disease was histologically minimal and did not increase with pro-

gression of the disease, it probably represents a systematic difference between the colonies and is not due to the respiratory disease.

There was no detectable difference in the functional residual capacities if animals of the same weight from the two colonies were compared. Comparing the animals at the same age showed that the functional residual capacity of the diseased animals are smaller because they weigh less at the same age.

Pulmonary compliance was not significantly different in the two colonies, but pulmonary resistance in tracheostomized animals showed an average increase of two and a half fold in the diseased animals.

These results suggest that the lungs have a large reserve and for functional disturbances to be detectable at rest, a large number of functional units must be involved. In endemic respiratory disease in the rat, pulmonary resistance was the first to show a detectable abnormality. This is in keeping with the clinical experience that disturbances in ventilatory function occur relatively early in human bronchitis and emphysema. In a mechanical system such as the lungs, it is perhaps to be expected that structural changes should first alter its mechanical properties.

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Note. Reference Nos. 73 and 99 have not been read in the original as these papers could not be obtained by the Library services.

A C K N O W L E D G E M E N T S

This thesis was prepared and written while the author held a joint appointment as Tutor and Registrar in the Department of Therapeutics, Queen's University, Belfast.

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TABLE I

Comparison of oxygen and carbon dioxide contents of human blood samples by the standard Van Slyke (1932) and Roughton and Scholander syringe (1943) methods.

Statistical analyses showed no significant difference between the methods.

OXYGEN CONTENT (vols. %)

VAN SLYKE	SYRINGE
12.6	11.9
18.8	17.8
19.3	19.0
15.7	15.8
18.7	18.3
12.5	12.5
16.4	16.4
17.1	17.5
20.0	20.8

Average Diff. = 0.12
t = 0.69
0.6 > P > 0.5

CO_2 CONTENT (vols. %)

VAN SLYKE	SYRINGE
44.1	46.3
49.7	49.3
52.2	51.4
56.3	54.0
46.2	43.3
75.0	73.3
51.8	52.5

Average Diff. = 0.74
t = 1.104
0.4 > P > 0.3

TABLE 2

Pilot study of oxygen saturation in 10 normal rats using tail blood samples. The mean value of 89.5% is in agreement with those obtained by Direct left ventricular puncture and oximetry.

10 NORMAL (S.P.F.) RATS - TAIL BLOOD

Oxygen content (vols. %)	Oxygen capacity (vols. %)	Oxygen saturation (%)
18.0	19.6	91.8
19.3	21.5	89.8
18.4	20.2	91.1
18.2	20.7	87.9
19.5	22.9	85.2
18.4	21.1	87.2
19.1	21.4	89.3
18.6	21.0	88.6
19.6	21.5	91.2
18.7	19.8	94.5
MEAN 18.8	21.0	89.5

Range of oxygen saturation = 85.2 - 94.5%

DIRECT L.V.P.

Oxygen content (vols. %)	Oxygen capacity (vols. %)	Oxygen saturation (%)
(14.57 (14.66	16.1	90.0
(13.21 (13.40	15.0	88.7

OXIMETRY

Oxygen saturation	..	..	.. =	88 % 92 %

TABLE 3

Comparison of oxygen contents of carotid and tail bloods from the same rat showing no statistical difference between the two samples.

The mean oxygen saturation and its range are similar to those in Table 2.

NORMAL (S.P.F.) RATS

Oxygen Contents vols. %		Oxygen capacity (vols. %)	Oxygen saturation (%)
Carotid blood	Tail blood		
19.5	19.7	21.7	91.2
19.5	19.0	21.6	90.3
17.6	18.2	21.0	86.2
19.9	19.1	21.4	92.1
20.6	20.7	22.3	93.3
16.3	16.2	18.5	88.6
18.1	17.9	19.4	93.8
19.0	18.8	21.9	87.2
Mean 18.8	18.7	21.0	90.3
Carotid blood v. Tail blood Average difference = 0.11 t = 0.6 0.6 > P > 0.5		Range of O_2 Saturation 86.2 - 93.8%	

TABLE 4

Data from analysis of tail blood samples of 35 normal (S.P.F.) rats.

	CO_2 Content (vols. %)	Oxygen content (vols. %)	Oxygen capacity (vols. %)	Oxygen saturation (%)
1	45.7	19.3	20.6	93.7
2	49.7	18.6	21.4	86.9
3	46.4	19.5	21.6	90.3
4	53.7	19.1	21.7	88.0
5	50.5	19.9	22.4	88.8
6	50.0	19.3	20.7	93.2
7	47.2	20.0	22.4	89.3
8	46.7	19.7	21.1	93.4
9	43.4	19.6	21.5	91.2
10	43.3	19.1	20.9	91.4
11	45.8	17.7	19.6	90.3
12	44.0	19.0	21.0	90.5
13	39.4	18.7	21.2	88.2
14	41.7	17.8	21.9	81.3
15	49.7	20.0	22.2	90.1
16	45.0	20.2	22.8	88.6
17	44.6	19.8	21.4	92.5
18	53.7	18.4	21.4	86.0
19	42.0	19.5	22.6	86.3
20	48.5	19.1	22.2	86.0
21	47.3	18.1	20.2	89.6
22	47.5	18.5	20.3	91.1
23	46.3	17.5	20.0	87.5
24	48.8	17.6	19.4	90.7
25	43.8	19.8	21.5	92.1
26	48.2	17.7	20.8	85.1
27	43.4	19.6	22.7	86.3
28	45.0	19.5	22.6	86.3
29	48.3	19.1	21.6	88.4
30	47.5	19.9	22.2	89.6
31	43.3	19.8	22.1	89.6
32	42.0	18.2	21.2	85.8
33	50.6	20.3	21.5	94.4
34	51.2	18.7	20.8	89.9
35	45.7	19.2	20.5	93.7
MEAN	46.6	19.08	21.4	89.3
Standard deviation	3.3	0.8	0.9	2.9
Standard error	0.56	0.14	0.15	0.49

TABLE 5

Effect on the oxygen contents of normal (S.P.F.) rats on breathing oxygen for 10 minutes. It can be seen that full saturation is not reached during oxygen breathing.

(The results have been corrected for dissolved oxygen.)

TAIL BLOOD SAMPLES

Oxygen Content (vols. %)		Oxygen Capacity (vols. %)
Before Oxygen Breathing	During oxygen Breathing	
18.7	19.8	20.8
19.3	19.8	21.5
19.1	19.1	21.0
19.2	20.0	22.6
18.9	20.0	21.4
19.4	19.9	22.4

TABLE 6

Comparison of blood gases (tail blood samples) between Normal (S.P.F.) and Diseased rats at two different age groups (six months and eighteen months).

NORMAL (S.P.F.) RATS

AGE - SIX MONTHS

	CO_2 Content (vols. %)	Oxygen Content (vols. %)	Oxygen Capacity (vols. %)	Oxygen Saturation (%)
1	47.5	19.6	21.9	89.5
2	43.7	18.7	20.7	90.3
3	43.6	18.9	22.5	84.0
4	48.7	20.2	20.7	97.6
5	48.3	20.5	22.1	92.8
6	47.3	18.9	20.9	90.4
7	49.9	19.3	21.4	90.2
8	46.2	18.3	21.2	86.3
9	46.2	20.2	21.8	92.7
10	47.1	19.1	21.2	90.1
11	45.5	18.7	21.2	88.2
12	47.3	19.2	21.8	88.1
MEAN	46.8	19.3	21.5	90.0

DISEASED RATS

	CO_2 Content (vols. %)	Oxygen Content (vols. %)	Oxygen Capacity (vols. %)	Oxygen Saturation (%)
1	53.3	16.6	19.9	83.4
2	49.5	17.6	19.9	88.4
3	51.0	17.6	19.4	90.7
4	46.4	18.3	21.9	83.6
5	48.5	18.6	21.3	87.3
6	42.4	18.9	21.9	86.3
7	46.4	18.0	20.4	88.2
8	49.1	18.7	21.0	89.0
9	44.3	18.9	22.1	85.5
10	49.3	18.1	21.4	84.6
11	47.2	18.6	21.3	87.3
12	42.0	18.5	21.4	86.4
MEAN	47.5	18.2	21.0	86.7

AGE - EIGHTEEN MONTHS

	CO_2 Content (vols. %)	Oxygen Content (vols. %)	Oxygen Capacity (vols. %)	Oxygen Saturation (%)
1	49.4	18.5	20.0	92.5
2	38.8	19.5	22.3	87.4
3	42.3	18.8	21.3	88.3
4	45.2	19.3	20.8	92.8
5	49.5	19.3	21.4	90.2
6	44.3	18.2	20.9	87.1
MEAN	44.9	18.9	21.1	89.7

	CO_2 Content (vols. %)	Oxygen Content (vols. %)	Oxygen Capacity (vols. %)	Oxygen Saturation (%)
1	52.2	18.0	20.7	87.0
2	45.3	17.6	21.1	83.4
3	50.5	18.4	20.8	88.5
4	48.9	19.1	22.6	84.5
5	46.8	18.6	21.0	88.6
6	47.7	18.3	22.6	81.0
7	52.2	18.5	21.0	88.1
8	36.4	18.1	21.0	86.2
MEAN	47.5	18.3	21.4	85.9

				Comparison of CO_2 Contents	Comparison of Oxygen Saturation
Six month rats.....	Normal	v.	Diseased ...	t = 0.7 0.5 > P > 0.4	t = 2.5 0.02 > P > 0.01*
Eighteen month rats	Normal	v.	Diseased ...	t = 1.0 0.4 > P > 0.3	t = 2.4 0.05 > P > 0.02*
Normal rats.....	6 Months	v.	18 Months ...	t = 1.4 0.2 > P > 0.1	t = 0.17 0.9 > P > 0.8
Diseased rats.....	6 Months	v.	18 Months ...	t = 0.02 1.0 > P > 0.9	t = 0.6 0.6 > P > 0.5

* Denotes differences that are statistically significant

TABLE 7

Functional Residual Capacities (F.R.C.) in 69 rats.

MALE

FEMALE

NORMAL (S.P.F. RATS)

Age (Months)	Weight (Grams)	F.R.C. (ml.)
2	193	2.65
3	296	3.78
5½	473	5.15
5½	345	3.42
5½	364	4.05
6	400	5.5
6	367	4.1
6	430	4.35
6	400	4.5
10	475	4.4
12	527	4.32
12	542	5.87
12	450	5.3
12	557	4.85
12	590	5.2
12	540	5.1
13	540	5.45
13	494	4.4
13	439	3.86
18	520	5.4
18	478	4.53
18	532	5.4
21	600	5.2
22	662	4.18

Age (Months)	Weight (Grams)	F.R.C. (ml.)
2	189	3.05
2	212	2.82
6	265	3.3
6	250	3.5
6	285	3.73
6	277	3.48
6	234	3.07
10	256	2.45
12	313	4.3
12	321	3.8
12	282	3.6
12	335	3.4
13	273	3.2
13½	346	3.6
18	380	3.15
18	306	3.56
18	430	3.18

DISEASED RATS

Age (Months)	Weight (Grams)	F.R.C. (ml.)
6	312	3.5
6	303	3.62
6	284	3.93
6	337	4.4
12	406	3.36
12	377	2.84
12½	469	4.9
12½	433	4.85
12½	410	4.75
18	425	4.23
18	452	4.1
27½	385	5.1

Age (Months)	Weight (Grams)	F.R.C. (ml.)
6	209	2.9
6	224	3.24
6	219	2.85
6	212	2.77
9	260	2.45
9½	259	2.08
10	277	2.35
10	278	3.28
10	300	2.7
11	251	3.5
11	280	3.15
12	240	3.7
12	271	2.83
12	252	2.3
18	255	3.5
18	226	3.2

TABLE 8

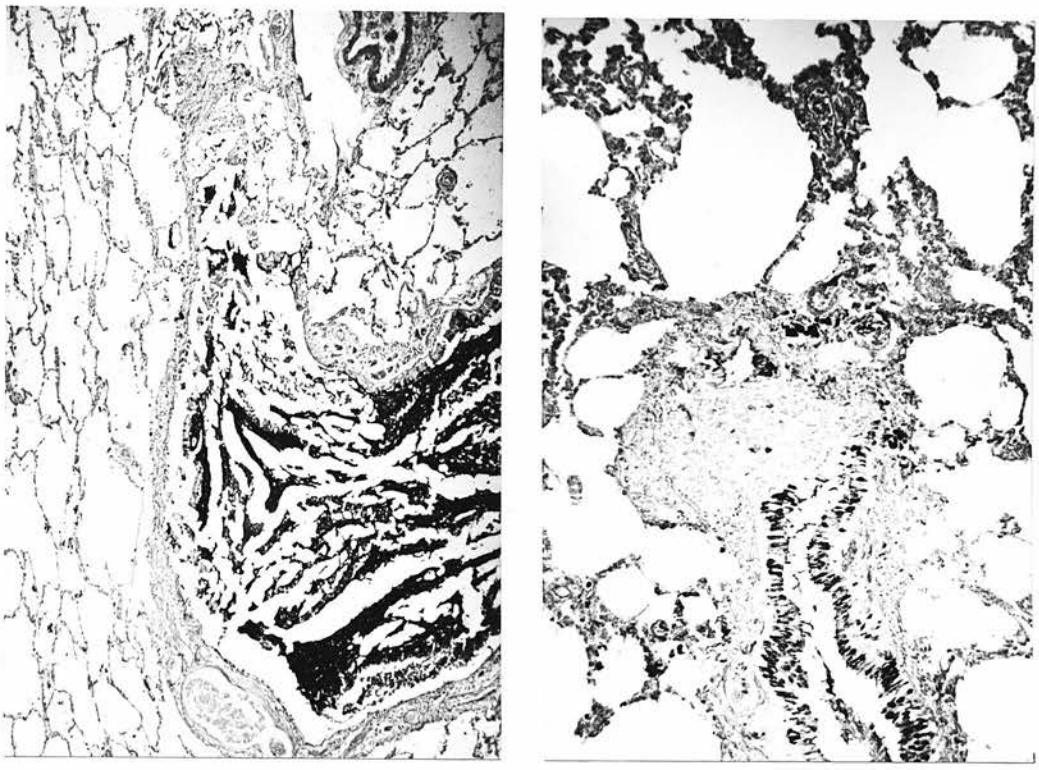
Comparison of Pulmonary Compliance and Flow Resistance between Normal (S.P.F.) and Diseased Rats

SEX	Weight Kilograms	Pulmonary Compliance ml./cm. H_2O	Pulmonary Resistance cm. H_2O/ml./sec. TOTAL	Pulmonary Resistance cm. H_2O/ml./sec. LOWER	Upper airway resistance % of total	Lower pulmonary resistance X Weight (Kg.)	Compliance / Weight (Kg.)
NORMAL (S.P.F.) RATS							
M	0.465	0.28	0.20	0.10	50	0.0465	0.605
M	0.432	0.26	0.09	-	-	-	0.601
M	0.540	0.34	0.09	-	-	-	0.629
F	-	0.23	0.18	-	-	-	-
M	-	-	0.15	-	-	-	-
M	0.304	0.22	0.20	-	-	-	0.723
M	0.354	0.26	0.10	-	-	-	0.734
F	0.290	0.21	0.32	0.19	41	0.0551	0.710
F	-	0.25	0.39	0.20	49	-	-
F	0.192	0.18	0.22	0.10	55	0.0192	0.937
F	0.211	0.28	0.22	0.07	68	0.0148	1.327
F	0.271	0.21	0.22	0.13	41	0.0352	0.775
F	0.264	0.24	0.24	0.08	67	0.0211	0.909
M	0.443	0.23	0.33	0.15	55	0.0664	0.519
M	0.383	0.26	0.28	0.13	54	0.0498	0.678
M	-	0.30	0.29	0.15	48	-	-
M	-	0.20	0.32	0.14	56	-	-
M	0.520	0.22	-	0.18	-	0.0936	0.423
M	0.478	0.30	-	0.21	-	0.1004	0.627
M	0.532	0.22	-	0.15	-	0.0798	0.413
MEAN	0.378	0.25	0.23	0.14	53	-	-
Standard Deviation	-	0.04	0.09	0.04	-	-	-
DISEASED RATS (< 12 months)							
F	0.250	0.13	0.36	0.18	50	-	-
F	0.245	0.12	0.40	0.15	62	-	-
F	0.248	-	0.39	-	-	-	-
M	0.398	0.21	0.32	0.13	59	-	-
M	0.312	0.20	0.29	-	-	-	-
M	0.375	0.24	0.26	0.15	42	-	-
M	0.304	0.25	0.13	0.06	54	-	-
M	0.278	0.29	0.23	-	-	-	-
DISEASED RATS (> 12 months)							
M	0.425	0.15	-	0.25	-	0.1062	0.353
M	0.452	0.21	-	0.35	-	0.1582	0.464
F	0.255	0.15	-	0.29	-	0.0739	0.588
F	0.226	0.22	-	0.30	-	0.0678	0.973
F	0.287	0.22	-	0.25	-	0.0717	0.766
F	0.270	0.13	-	0.42	-	0.1134	0.481
M	0.448	0.21	-	0.43	-	0.1926	0.468

Total Pulmonary Resistance Normal v. Diseased (< 12 months) $t = 1.88$ $0.10 > P > 0.05$

	Comparison of Compliance	Comparison of Lower Pulmonary Resistance
Normal Rats v. Diseased Rats (< 12 months)	$t = 1.98$ $0.10 > P > 0.05$	$t = 0.32$ $0.8 > P > 0.7$
Normal Rats v. Diseased Rats (> 12 months)	$t = 3.5$ $0.01 > P > 0.001$*	$t = 7.3$ $P < .001$*
Diseased Rats (< 12 months) v. Diseased Rats (> 12 months)	$t = 0.77$ $0.5 > P > 0.4$	$t = 5.1$ $P < .001$*
AFTER CORRECTION FOR WEIGHT		
Normal Rats v. Diseased rats (> 12 months)	$t = 1.05$ $0.4 > P > 0.3$	$t = 3.9$ $.01 > P > .001$*

* Denotes differences that are statistically significant



(a) Human Lung (b) Rat Lung

Figure 1

Microphotographs to show the histological similarity between human bronchitis and endemic bronchial disease in the rat. Both preparations depict a terminal bronchus with free mucus in the lumen, goblet cells in the bronchial wall and centrilobular emphysema. The only difference is the presence of lymphoid hyperplasia in the rat.

(a) Human lung fixed with formal saline under 10 cm. water pressure intrabronchially.
Stain : Periodic acid schiff. x 32

(b) Rat lung fixed intratracheally with Carnoy's fluid.
Stain : Periodic acid schiff. x 80

Figures 2, 3 and 4.

Whole lung sections from rats to show healthy lungs in the S.P.F. colony and progression of respiratory disease with age in the diseased colony.

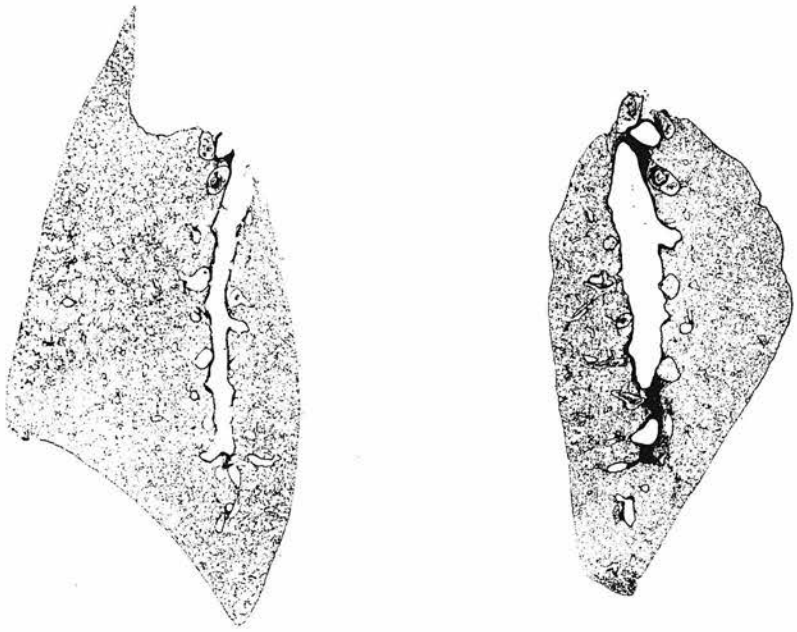
Lungs fixed by intratracheal Carnoy's fluid.
Stained Haematoxylin x 3.

S.P.F. Rat

Diseased Rat

Figure 2

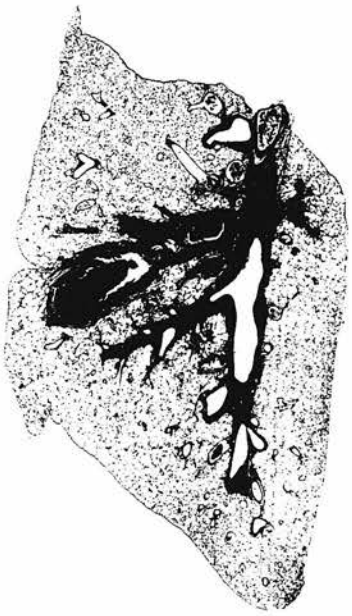
Six-month rats. Whole lung sections showing minimal bronchial disease in the diseased rat.



S.P.F. Rat Diseased Rat

Figure 3

Twelve-month rats. Whole lung sections showing obvious bronchial disease in the diseased rat.



S.P.F. Rat

Diseased Rat

Figure 4

Eighteen-month rats. Whole lung sections showing marked bronchial disease in the diseased rat. Note absence of disease in the S.P.F. rat even at this age.

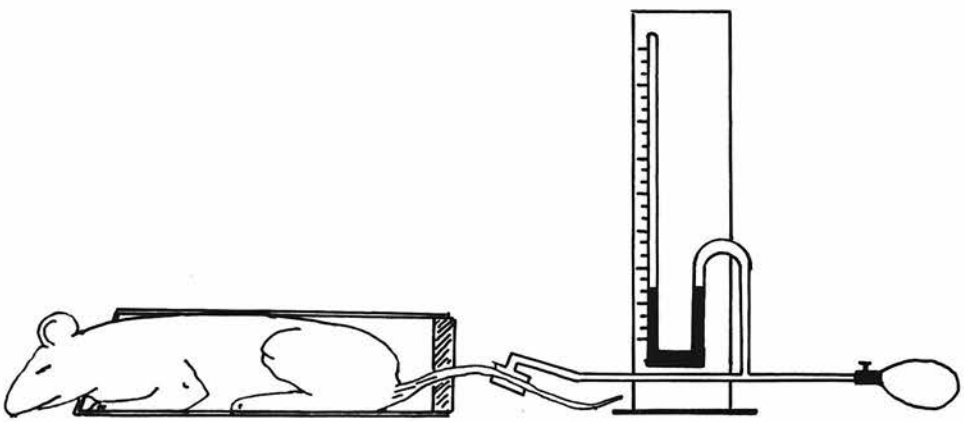


Figure 5

Method of obtaining tail blood samples from the rat.

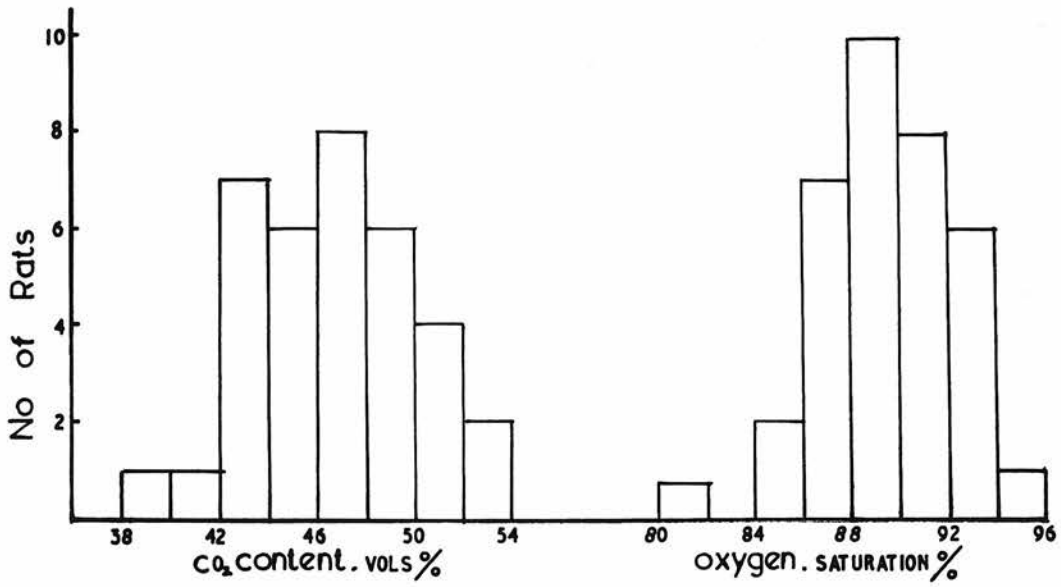


Figure 6

Histogram of blood gas data from 35 normal (S.P.F.) rats to show normal distribution of results.

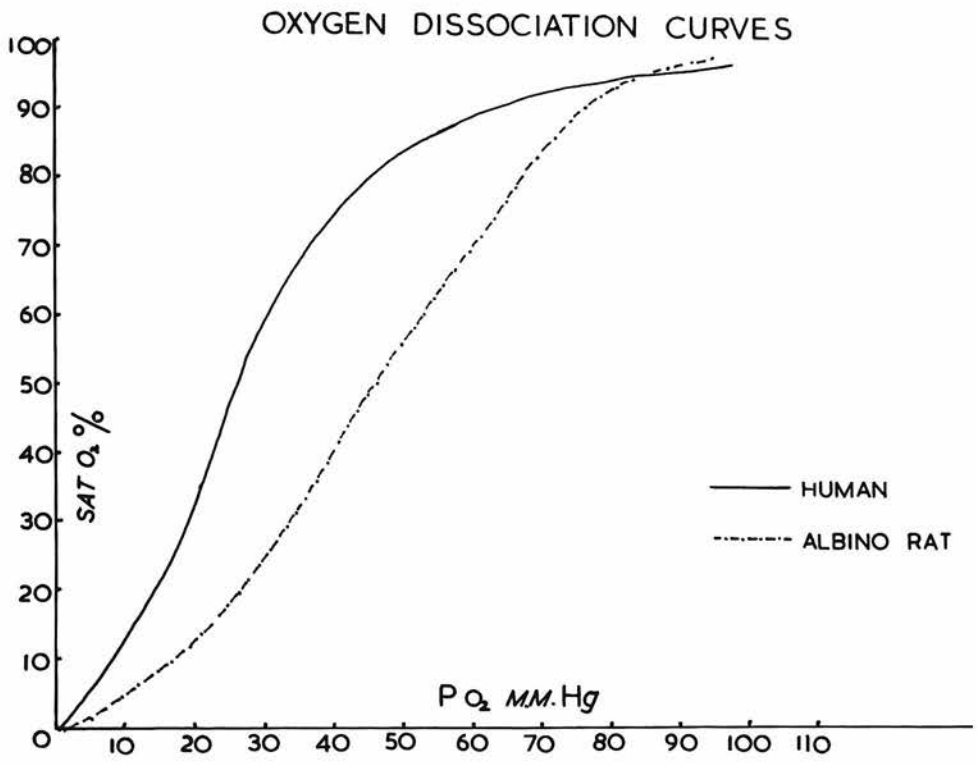


Figure 7

Human and Rat blood oxygen dissociation curves compared.
Human curve from Dill (1944) Temp. 37°C pH = 7.4
Rat curve from Jones et. al. (1950) Temp. 38°C pH = 7.4
(according to curves, at oxygen pressure of 40 mm. Hg. blood oxygen saturation is 75% in human and 40% in rat - see text).

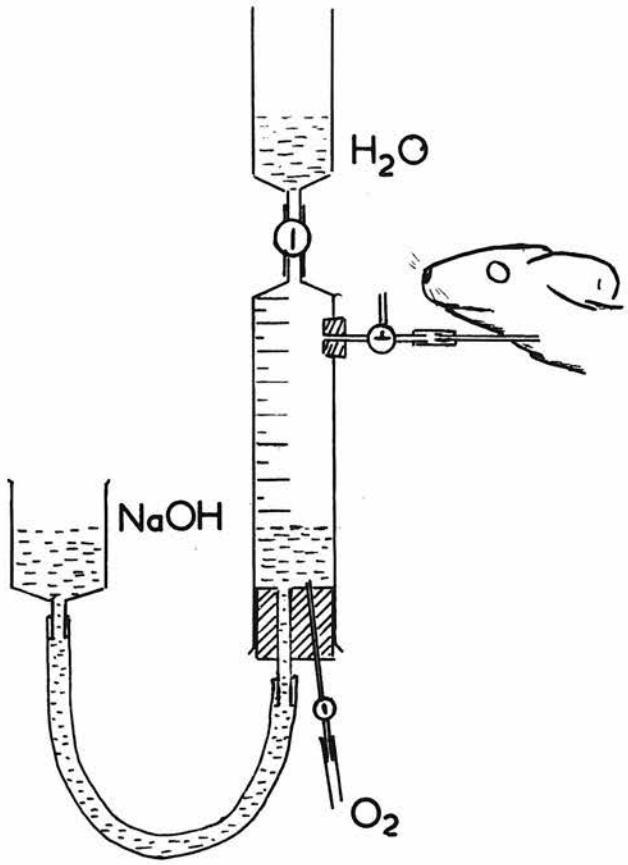
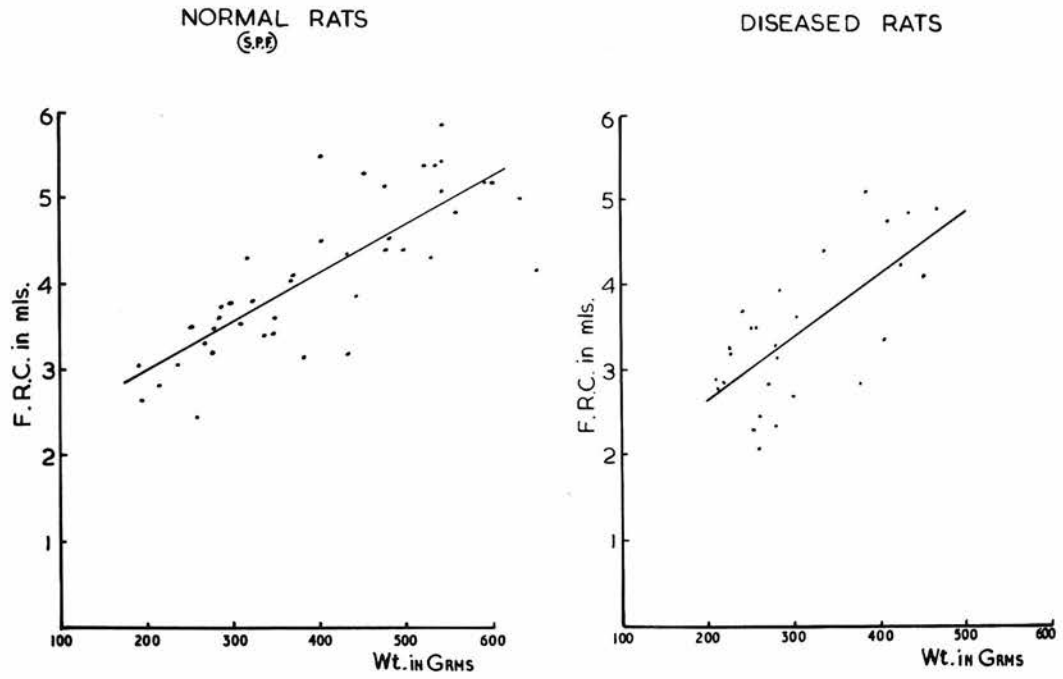


Figure 8

Method of measuring lung volume (F.R.C.) in the rat.



$r = 0.8 \quad P < 0.001$

$b = 0.0057$

$r = 0.7 \quad P < 0.001$

$b = 0.0073$

Figure 9

Relationship between lung volume (F.R.C.) and body weight in rats. Solid lines represent calculated regression lines. There is a high degree of correlation between lung volume and body weight in both colonies.

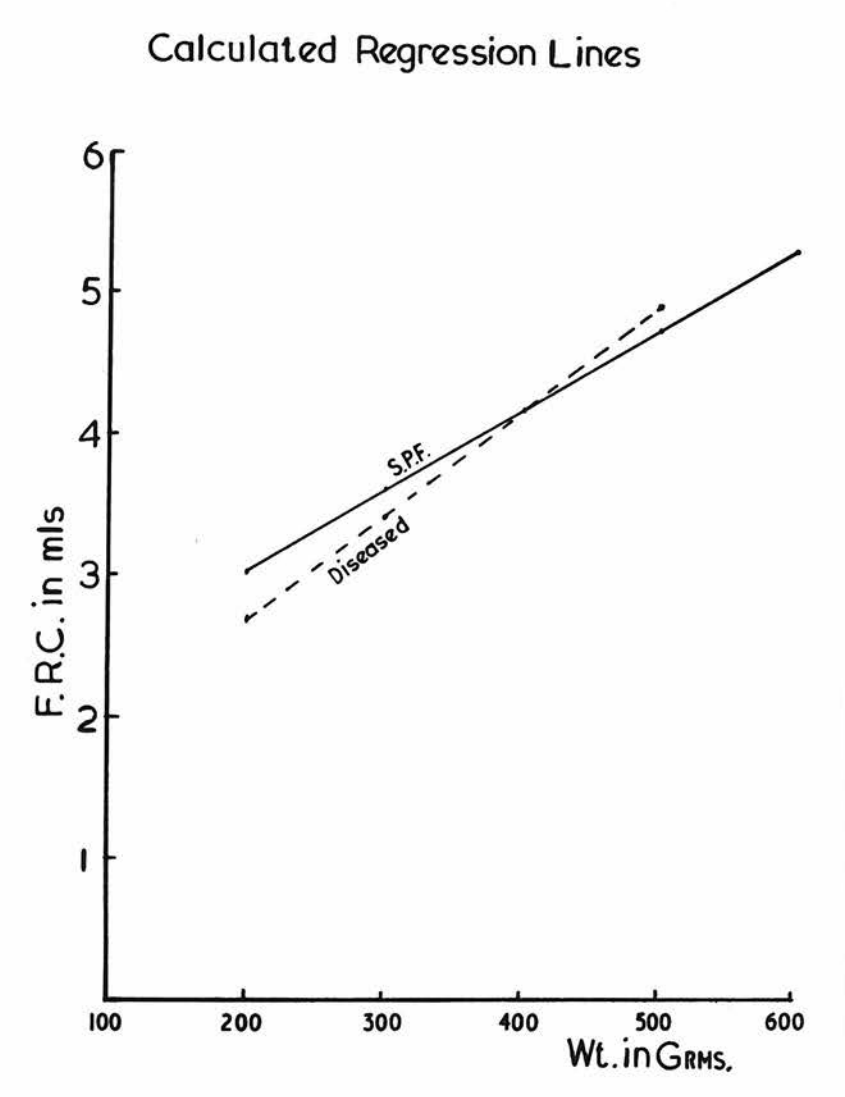


Figure 10

Relationship between lung volume (F.R.C.) and body weight. To show close approximation of calculated regression lines from the S.P.F. and diseased colonies.

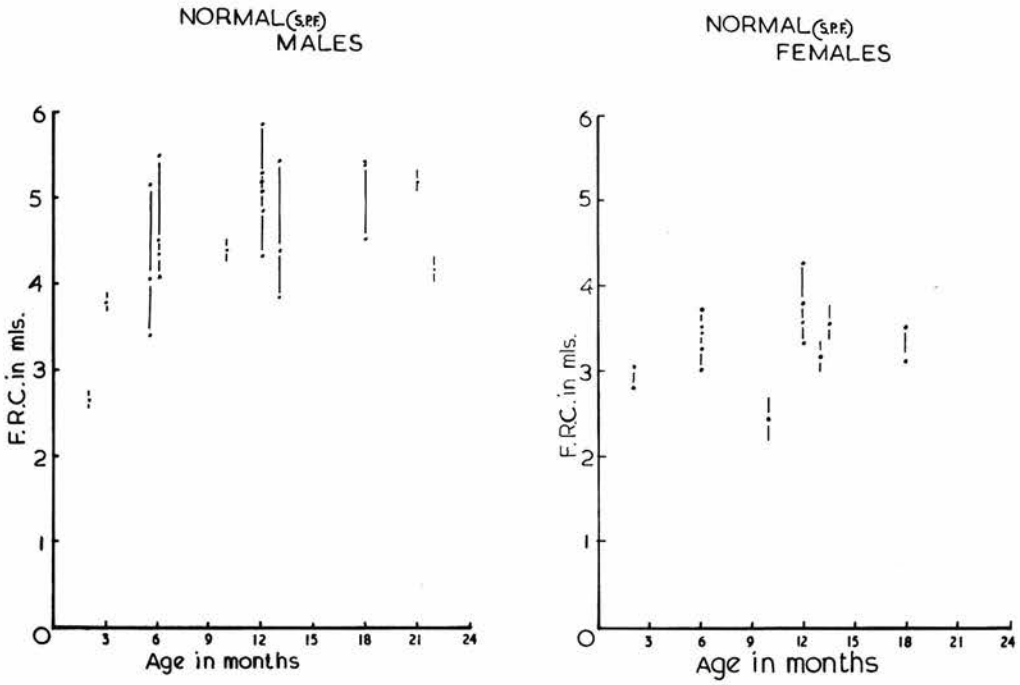


Figure 11

Relationship between lung volume (F.R.C.) and age.

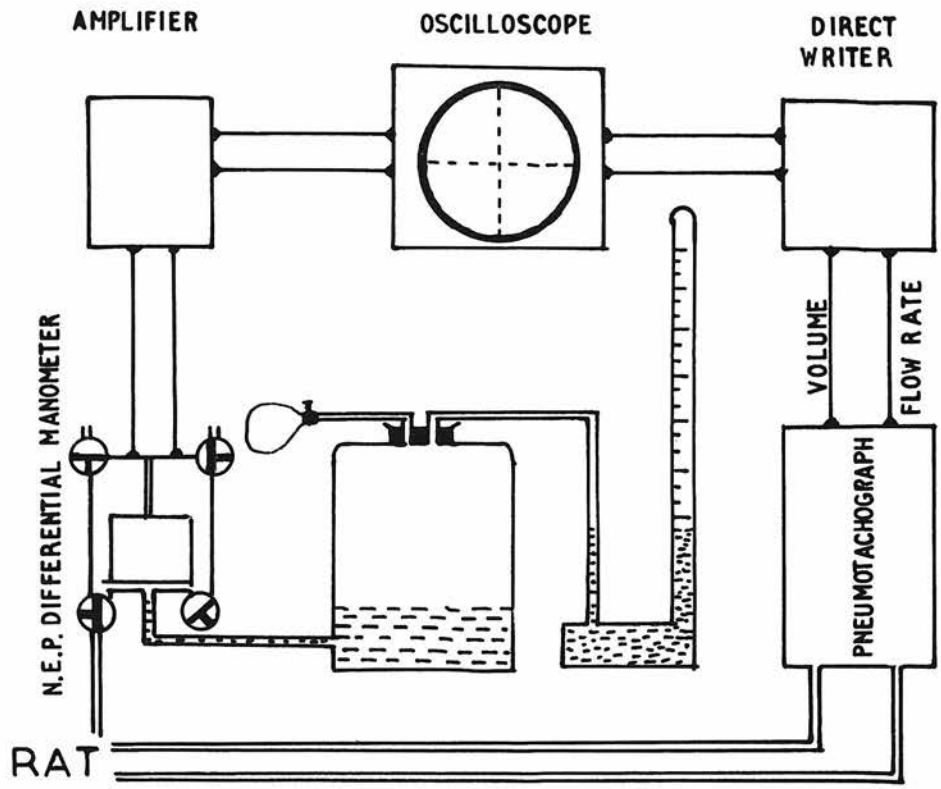


Figure 12

Arrangement of apparatus for measurement of mechanical properties of lungs.

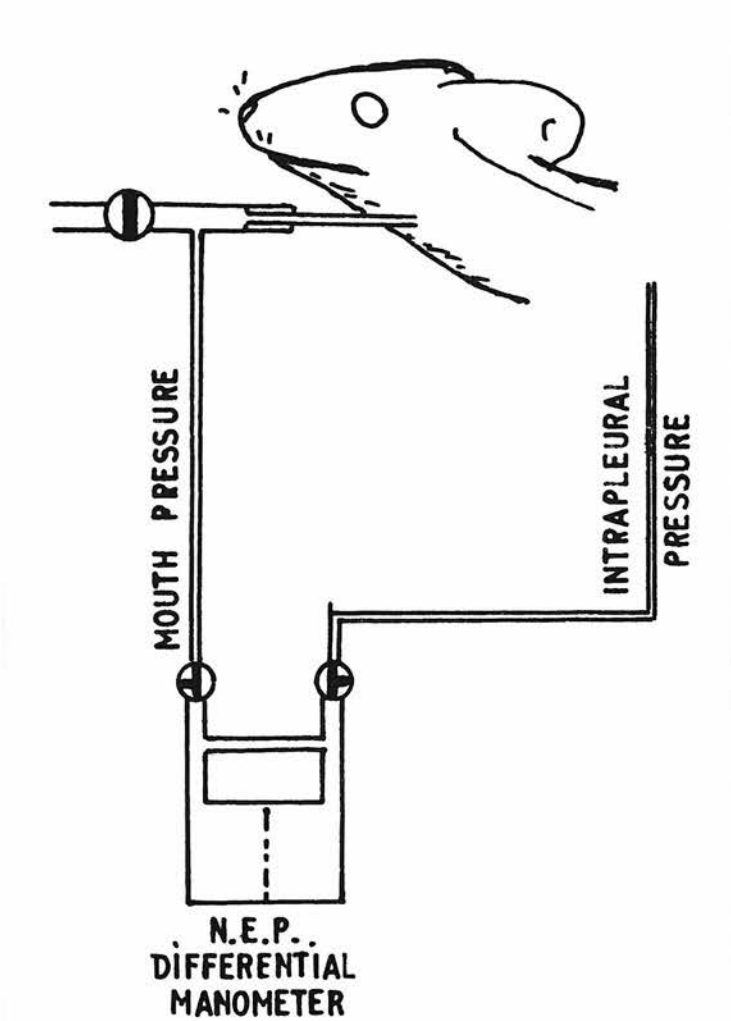


Figure 13

Method of measuring transpulmonary pressure during respiratory obstruction in pulmonary compliance determinations.

$$C = \frac{\Delta V}{\Delta P}$$

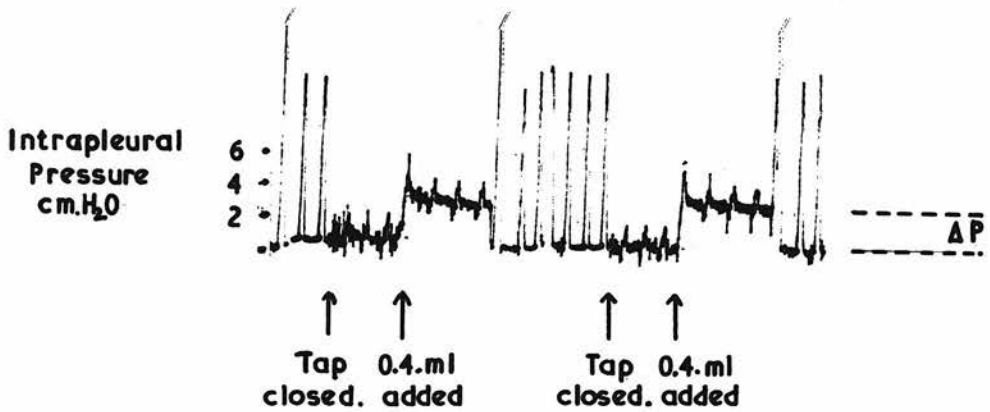


Figure 14

Sample of respiratory tracing and method of measuring pulmonary compliance. ΔP = change in transpulmonary pressure.

$$R = \frac{\Delta P}{\Delta \dot{V}}$$

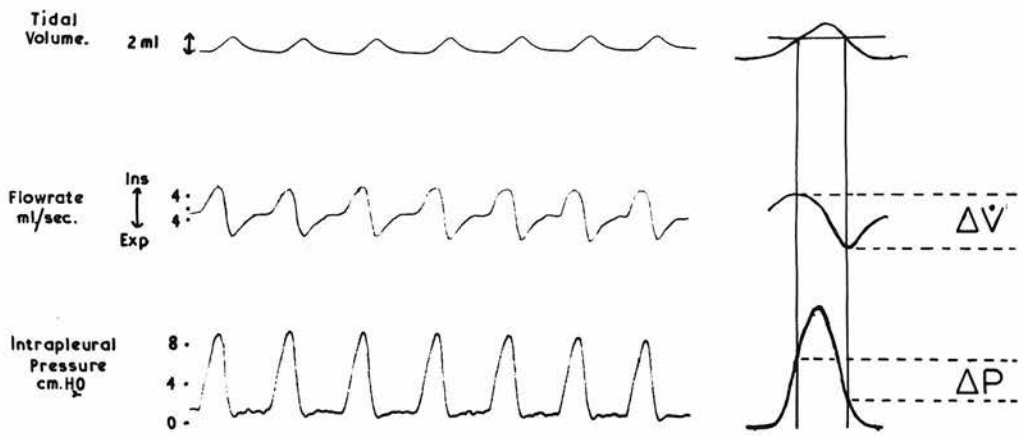


Figure 15

Sample of respiratory tracing and method of analysis for pulmonary resistance.